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**Request
for
Continued Examination (RCE)
Transmittal**

Address to:
Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Application Number	10/646,682
Filing Date	08/22/2003
First Named Inventor	Dennis S. Fernandez
Art Unit	1631
Examiner Name	DeJong, Eric S.
Attorney Docket Number	FERN-P013

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. See Instruction Sheet for RCEs (not to be submitted to the USPTO) on page 2.

1. Submission required under 37 CFR 1.114 Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

a. Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

i. Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____

ii. Other _____

b. Enclosed

i. Amendment/Reply iii. Information Disclosure Statement (IDS)

ii. Affidavit(s) / Declaration(s) iv. Other Copies of 6 foreign patents, 102 IPL refs _____

2. Miscellaneous

a. Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of _____ months. (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

b. Other _____

3. Fees The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

The Director is hereby authorized to charge the following fees, any underpayment of fees, or credit any overpayments, to Deposit Account No. 500482.

i. RCE fee required under 37 CFR 1.17(e)

ii. Extension of time fee (37 CFR 1.136 and 1.17)

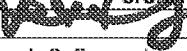
iii. Other _____

b. Check in the amount of \$ _____ enclosed

c. Payment by credit card (Form PTO-2038 enclosed)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

Signature		Date	7/23/2009
Name (Print/Type)	Dennis S. Fernandez	Registration No.	34,160

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450 or facsimile transmitted to the U.S. Patent and Trademark Office on the date shown below.

Signature		Date	
Name (Print/Type)			

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: Dennis S. Fernandez Attorney Docket No.: FERN-P013

Serial No.: 10/646,682 Group Art Unit: 1631

Filed: 08/22/2003 Examiner: Eric S. Dejong

Confirmation No.: 1019

Title: Integrated Biosensor and Simulation System for Diagnosis and Therapy

PRELIMINARY AMENDMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

This responds via RCE-application to FINAL Office Action dated 05/27/09.

Listing of Claims:

1-35. (Cancelled)

36. (CURRENTLY AMENDED) A systems-biology platform-based integrated biosensor and simulation system comprising:

at least one implantable biosensor for sensing a biological target to generate a signal; and

a simulator comprising a systems-biology platform for using the signal and a model of the target to generate a therapeutic or diagnostic output;

wherein said sensor is reconfigurable by said simulator, whereby the simulator automatically integrates the target biosensing with in-vivo modeling to simulate the biological target as a whole organism using the systems-biology platform that comprises genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics, such reconfiguration thereby reconfiguring a biocatalytic chip, a logic device, a tissue scaffold, a therapeutic reservoir, a probe arranger, or a DNA microarray.

37. (Previously presented) The system of claim 36 wherein: the sensor senses a food material for consumption by the biological target to generate a second signal, the simulator further using the second signal to generate the therapeutic or diagnostic output.

38. (Previously presented) The system of claim 36 wherein: the simulator generates the output according to a regulatory condition.

39. (Previously presented) The system of claim 36 wherein: the sensor couples to the simulator via a programmable switch.

40. (CURRENTLY AMENDED) A systems-biology platform-based method comprising the steps of:

sensing with an implantable biosensor a biological target to generate a signal; and

simulating with a simulator comprising a systems-biology platform using the signal and a model of the target to generate a therapeutic or diagnostic output; wherein said simulator reconfigures said biosensor, whereby the simulator automatically integrates the target biosensing with in-vivo modeling to simulate the biological target as a whole organism using the systems-biology platform that comprises genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics, such reconfiguration thereby reconfiguring a biocatalytic chip, a logic device, a tissue scaffold, a therapeutic reservoir, a probe arranger, or a DNA microarray.

41. **(Previously presented)** The method of claim 40 wherein: the sensor senses a food material for consumption by the biological target to generate a second signal, the simulator further using the second signal to generate the therapeutic or diagnostic output.
42. **(Previously presented)** The method of claim 40 wherein: the simulator generates the output according to a regulatory condition.
43. **(Previously presented)** The method of claim 40 wherein: the sensor couples to the simulator via a programmable switch.
44. **(Previously presented)** The method of claim 40, wherein said sensor is implanted in a subject's mouth, larynx, blood vessel, vein, nose, ear, eye, heart, brain, lymph node, lung, breast, stomach, pancreas, kidney, colon, rectum, ovary, uterus, bladder or prostate.
45. **(Previously presented)** The method of claim 40, wherein said biosensor comprises an array of at least two sensors.

46. **(Previously presented)** The method of claim 45, wherein said at least two sensors are capable of sensing two different biological targets.

47. **(Previously presented)** The method of claim 46, wherein said different biological targets are selected from a group consisting of DNA, RNA, peptide, antibody, antigen, tissue factor, virus, lipid, fatty acid, steroid, neurotransmitter, carbohydrate, free radical, neural, chemical, metabolite and cell.

48. **(Previously presented)** The method of claim 40, wherein said reconfiguring comprises activating or deactivating said biosensor.

49. **(Previously presented)** The method of claim 45, wherein said reconfiguring comprises activating or deactivating at least one of said at least two sensors.

50. **(Withdrawn)** The method of claim 40, wherein said reconfiguring comprise hardware reconfiguration.

51. **(Previously presented)** The system of claim 36, wherein said simulator is capable of activating or deactivating said sensor.

52. **(Previously presented)** The system of claim 36, wherein said sensor is capable of functioning in a subject's mouth, larynx, blood vessel, vein, nose, ear, eye, heart, brain, lymph node, lung, breast, stomach, pancreas, kidney, colon, rectum, ovary, uterus, bladder or prostate.

53. **(Previously presented)** The system of claim 36, wherein said biosensor comprises said at least one sensor and at least a second sensor.

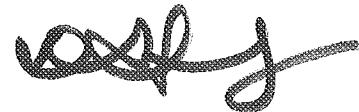
54. (Previously presented) The system of claim 53, wherein said at least one sensor and said at least second sensor are capable of sensing two different biological targets.

55. (Previously presented) The systems of claim 54, wherein said different biological targets are selected from a group consisting of DNA, RNA, peptide, antibody, antigen, tissue factor, virus, lipid, fatty acid, steroid, neurotransmitter, carbohydrate, free radical, neural, chemical, metabolite and cell.

REMARKS

Re 35. USC.103a Examiner rejection of claims 36-49 and 51-55 over Porat and Giuffre, Applicant amends claims to specify that the systems-biology platform comprises "genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics."

Respectfully submitted,



Dennis S. Fernandez, ESQ.
Reg. No. 34,160

Date: 7/23/2009

Address: **FERNANDEZ & ASSOCIATES, LLP**
Patent Attorneys

Customer No: 22877

Phone: (650) 325-4999
Fax: (650) 325-1203
Email: iploft@iploft.com

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: **Dennis S. Fernandez** Attorney Docket No.: **FERN-P013**
Serial No.: **10/646,682** Group Art Unit: **1631**
Filed: **08/22/2003** Examiner: **DeJong, Eric S**
Title: **Integrated biosensor and simulation system for diagnosis and therapy**
Confirmation No. **1019**

AMENDMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

This responds to Office Action dated 9/2/2009.

Listing of Claims:

1-35. (Cancelled)

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at least one implantable biosensor for sensing a biological target to generate a signal; and

a simulator comprising a systems-biology platform for using the signal and a model of the target to generate a therapeutic or diagnostic output;

wherein said sensor is reconfigurable by said simulator, whereby the simulator automatically integrates the target biosensing with in-vivo modeling to simulate the biological target as a whole organism using the systems-biology platform that comprises computational modeling hardware and software analysis genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics automated tools, such reconfiguration thereby reconfiguring a biocatalytic chip, a logic device, a tissue scaffold, a therapeutic reservoir, a probe arranger, or a DNA microarray.

37. (Previously presented) The system of claim 36 wherein: the sensor senses a food material for consumption by the biological target to generate a second signal, the simulator further using the second signal to generate the therapeutic or diagnostic output.

38. (Previously presented) The system of claim 36 wherein: the simulator generates the output according to a regulatory condition.

39. (Previously presented) The system of claim 36 wherein: the sensor couples to the simulator via a programmable switch.

40. (CURRENTLY AMENDED) A systems-biology platform-based method comprising the steps of:

sensing with an implantable biosensor a biological target to generate a signal; and

simulating with a simulator comprising a systems-biology platform using the signal and a model of the target to generate a therapeutic or diagnostic output; wherein said simulator reconfigures said biosensor, whereby the simulator automatically integrates the target biosensing with in-vivo modeling to simulate the biological target as a whole organism using the systems-biology platform that comprises computational modeling hardware and software analysis genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics automated tools, such reconfiguration thereby reconfiguring a biocatalytic chip, a logic device, a tissue scaffold, a therapeutic reservoir, a probe arranger, or a DNA microarray.

41. (Previously presented) The method of claim 40 wherein: the sensor senses a food material for consumption by the biological target to generate a second signal, the simulator further using the second signal to generate the therapeutic or diagnostic output.

42. (Previously presented) The method of claim 40 wherein: the simulator generates the output according to a regulatory condition.

43. (Previously presented) The method of claim 40 wherein: the sensor couples to the simulator via a programmable switch.

44. (Previously presented) The method of claim 40, wherein said sensor is implanted in a subject's mouth, larynx, blood vessel, vein, nose, ear, eye, heart, brain, lymph node, lung, breast, stomach, pancreas, kidney, colon, rectum, ovary, uterus, bladder or prostate.

45. **(Previously presented)** The method of claim 40, wherein said biosensor comprises an array of at least two sensors.

46. **(Previously presented)** The method of claim 45, wherein said at least two sensors are capable of sensing two different biological targets.

47. **(Previously presented)** The method of claim 46, wherein said different biological targets are selected from a group consisting of DNA, RNA, peptide, antibody, antigen, tissue factor, virus, lipid, fatty acid, steroid, neurotransmitter, carbohydrate, free radical, neural, chemical, metabolite and cell.

48. **(Previously presented)** The method of claim 40, wherein said reconfiguring comprises activating or deactivating said biosensor.

49. **(Previously presented)** The method of claim 45, wherein said reconfiguring comprises activating or deactivating at least one of said at least two sensors.

50. **(Withdrawn)** The method of claim 40, wherein said reconfiguring comprise hardware reconfiguration.

51. **(Previously presented)** The system of claim 36, wherein said simulator is capable of activating or deactivating said sensor.

52. **(Previously presented)** The system of claim 36, wherein said sensor is capable of functioning in a subject's mouth, larynx, blood vessel, vein, nose, ear, eye, heart, brain, lymph node, lung, breast, stomach, pancreas, kidney, colon, rectum, ovary, uterus, bladder or prostate.

53. **(Previously presented)** The system of claim 36, wherein said biosensor comprises said at least one sensor and at least a second sensor.
54. **(Previously presented)** The system of claim 53, wherein said at least one sensor and said at least second sensor are capable of sensing two different biological targets.
55. **(Previously presented)** The systems of claim 54, wherein said different biological targets are selected from a group consisting of DNA, RNA, peptide, antibody, antigen, tissue factor, virus, lipid, fatty acid, steroid, neurotransmitter, carbohydrate, free radical, neural, chemical, metabolite and cell.

REMARKS

Re Information Disclosure Statement Examiner annotation that “copies of cited art not provided”, applicant respectfully submits that copies of such cited foreign patent documents were in-fact provided previously, as evidenced properly in PAIR (see attached listing of PAIR image file wrapper with said foreign references indicated).

To overcome 35.USC.112 1st and 2nd-paragraph rejection of claims 36-49 and 51-55, applicant amends independent claims 36 and 40 to particularly point out and distinctly claim the subject matter which applicant regards as the invention, as well as comply with written description requirement, particularly specifying structural and functional limitation (i.e., not merely abstract scientific disciplines) such that systems-biology platform (Fig.3a) comprises “computational modeling hardware and software analysis” genomics, proteomics (para.124), computational chemistry (para.125), pharmacogenomics (para.126), computational biology, computational biophysics, computational cell behavior (para.127), pharmacokinetics (para.128), metabolomics (para.129), and transcriptomics (para.130) automated tools, whereby such computational modeling hardware and software analysis automated tools are described in specification pages 36-38 in such a way to reasonably convey to one of skilled in the relevant art that inventor at the time the application was filed had possession of the claimed invention.

Further to overcome 35.USC.103a rejection of claims 36-49 and 51-55 over Porat and Giuffre, applicant respectfully submits that such cited prior art neither teach, suggest nor motivate in any predictable or common sense “systems-biology platform” comprising

computational modeling hardware and software analysis genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics automated tools, as required by applicant's invention now claimed herein.

Respectfully submitted,



Dennis S. Fernandez, ESQ.
Reg. No. 34,160

Date: 10/05/09

Address: **FERNANDEZ & ASSOCIATES, LLP**
Patent Attorneys

Customer No: 22877

Phone: (650) 325-4999
Fax: (650) 325-1203
Email: iploft@iploft.com

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Application

For

10 *United States Non-Provisional Utility Patent*

Title:

Integrated Biosensor and Simulation System for Diagnosis and Therapy

15

Inventor:

20 DENNIS SUNGA FERNANDEZ, residing at 1175 Osborn Avenue, Atherton, CA 94027, citizen of United States of America.

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Integrated Biosensor and Simulation System for Diagnosis and Therapy

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BACKGROUND

Field of Invention

Invention relates to sensors and related software for monitoring or analyzing
10 biological hosts or material.

10

Related Background Art

Various sensors are used to detect or measure macroscopic or molecular
physiology in humans or other biological host. Additionally systems-biology software
15 provides computational modeling of molecular structures and interactions for genomics,
proteomics, metabolomics, transcriptomics, computational chemistry,
pharmacogenomics, or other purpose. Such tools, however, are not easily or
automatically integrated or reconfigurable for interdisciplinary diagnosis or therapy.

SUMMARY

20 Integrated biosensor-simulation system combines one or more sensor to detect
various conditions in biological target or host, and software program or simulator using
system-biology model and sensor data adaptively to provide therapy, diagnosis, or other
automated feedback. Preferably one or more sensor is reconfigurable by the simulator.
Optionally food material for consumption by the biological target is sensed for
25 application to the simulator, which may apply certain regulatory condition. Switch
couples simulator programmably to sensors.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1a shows sensor device according to aspect of present invention.

Figure 1b shows sensor network according to aspect of present invention.

Figure 2 shows sensor system according to aspect of present invention.

5 Figure 3a shows systems-biology software according to aspect of present invention.

Figure 3b shows systems-biology software and data according to aspect of present invention.

10 Figure 3c shows system biology software and sensor according to aspect of present invention.

Figure 4a shows system biology software according to aspect of present invention.

Figure 4b shows therapy according to aspect of present invention.

Figure 4c shows therapy reservoir according to aspect of present invention.

15 Figure 4d shows sensor reconfiguration according to aspect of present invention.

Figure 5 shows DNA sensor according to aspect of present invention.

Figure 6 shows diagnosis or therapy method according to aspect of present invention.

DETAILED DESCRIPTION

Figure 1a architectural diagram illustrates implantable network biosensor 100. It is contemplated herein that sensor 100 may also operate without being implanted in biological host, but instead through external contact or attachment thereto. Optionally 5 multiple coupled sensors 100 may provide fault-tolerant back-up or recovery facility, in case one or more sensors fails or malfunctions. Sensor 100 may be provided inside a host, e.g., mouth, larynx, blood vessel, vein, nose, ear, eye, heart, brain, lymph node, lung, breast, stomach, pancreas, kidney, colon, rectum, ovary, uterus, bladder, prostate, or other organ or using portable mobile application externally, e.g. skin, fingernail.

10

Sensor 100 includes sensor unit 111, controller 112, therapeutic unit 113, and power module 114. Sensor 100 components may be interconnected or communicate with other components using electrical, electronic, or electromagnetic signals, e.g., optical, radio frequency, digital, analog or other signaling scheme. Power module 114 provides 15 electrical energy for sensor 100 to operate.

Generally biosensor 100 may sense individual genome, proteome, metabolism, transcription, translation, blood pressure, carbohydrate and oxygen concentrations, or other factors as described herein. Data is provided by sensor 100 to integrated network 20 103 that applies systems-biology software 104 to verify, model, or analyze, for example, relative sequences, 3-dimensional structure, molecular interactions, or overall cellular and physiological environment.

Systems-biology software 104 processes information and determines treatment dynamically for individual real-time physiological condition. Analysis report and other patient instructions are transmitted remotely as telemedicine service to network 103, which provides tasks to components, such as pharmaceutical or biopharmaceutical 5 reservoirs 109, reconfigurable biosensors 102, wireless telemetry system 106, therapeutic manufacturers 108, or other applications.

Sensors 102 may be hardware-reconfigurable or software-programmable according to user or systems-biology programming or report instructions. Ongoing or 10 intermittent scheduled or random sensing events occurs between therapeutic components and pre-programmed and reconfigured micro/nano biosensors 102, along with proactive or reactive feedback to patient or user from systems-biology platform 104. Preferably sensing process employs micro or nanoscale sensor 102 structure for minimal intrusion to individual health or physiology.

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Optionally sensor system 100 provide wireless (RF) signal coupling with other sensors 100, such that communication occurs between different organisms having sensor 100. For example, sensor 100 may be implanted in pregnant host and another sensor 100 implanted in such host baby. Communication between sensors 100 may provide effective 20 biological sensor signal transmission between separate hosts or organisms. Sensor 100 may be accessible according to IEEE 1451 network interface format.

Another example for multi-host communication implements sensors 100 for communication between separate related individuals, such as potential sexual partners, where one partner sensor 100 may sense sexually transmitted disease (STD) in such host, then such information is provided electronically to other host sensor 100 to produce 5 proper antigens and antibodies to combat the STD.

Sensor unit 111 uses positioning device or chip 101 to position, locate or immobilize effectively target sample for analysis or sensing. The manipulated targeted sample comprises a biological molecule, organic or inorganic substance, such as cells, 10 tissue, nutrients, chemicals, intracellular materials, extra-cellular materials, charged ions, pharmaceuticals, or molecular materials affecting host physiology.

Sensor unit 111 comprises multifunctional biosensor platform 102 for sensing and monitoring multiple biological materials, concentrations, inorganic or organic materials, 15 cellular material, genetic material, nucleic acids, proteins, amino acids, peptides, antibodies, antigens, fatty acids, lipids, steroids, neurotransmitters, inorganic ions, pH levels, free radicals, carbohydrates, chemicals, small molecules, cells, tissue, pharmaceuticals, toxins, metabolites, or physiological levels macroscopically, microscopically, or nanoscopically.

20

Controller 112 uses network 103 to couple components for signal or data communication. Network 103 communicates data electronically to systems-biology platform 104. Controller 112 may be implemented using personal, desktop, server,

notebook, mainframe, wireless portable or other computer or processing device having processor, digital memory and network or user interface.

Systems-biology platform 104 uses computer equipment, software programs or reconfigurable firmware or emulation logic devices to verify, model, simulate, or analyze 5 stored or raw data using computational biology, such as bioinformatics, proteomics, metabolomics, pharmacogenomics or other analysis software or hardware tools. Systems-biology platform 104 interprets or integrates data from biosensor platform 102, and analyzes organism preferably as a whole on system level. Systems-biology platform 104 may be integrated within one or more integrated circuit, module or processor; or 10 bilaterally communicate to outside non-host signal source through wireless communication unit 106.

Controller 112 may use data storage 105 for storing processed data or applications programs from systems-biology platform 104. Controller 112 includes wireless communication unit 106, allowing bilateral communication with outside source, which 15 may access or control sensor unit 111, controller 112, or therapeutic unit 113 through wireless communication unit 106.

Network 103 may couple therapeutic unit 113 with controller unit 112. Therapeutic unit 113 includes therapeutic manufacture 108 for providing pharmaceuticals, biopharmaceuticals, bio-catalytic chips or devices, tissue, or 20 physiological treatments. Biopharmaceuticals include biological material for therapeutic use.

Therapeutic unit 113 includes therapeutic reservoir 109, which provides micro or nano-scale reservoirs containing pharmaceuticals or biopharmaceuticals. Contents of therapeutic reservoirs 109 may be provided or configured before sensor 100 is implanted in or attached to organism, or may be manufactured and filled *in vivo* by therapeutic manufacture 108. Therapeutic reservoirs 109 may release or dispense contents when appropriately signaled by network 103.

Therapeutic unit 113 includes sensor manufacture 110 unit, which may provide additional sensors *in vivo* for additional targeted sensing or monitoring. Sensors from sensor manufacture 110 are part of or comprise biosensor platform 102.

Figure 1b shows positioning chip 101 for immobilizing or positioning target or tissue samples on or in sensor 102 for bio-sensing as described herein. Positioning chip 101 may use micro-fabrication, micro-fluidics, or microbiology to manipulate, sort, or prepare samples, reagents, or other biological entities for analysis, high-throughput assays, or diagnostic applications. Positioning chip 101 may accomplish sample placement using multi-channel patch clamp electrophysiology chip to control individual cells by applying current to cell ion channels, positioning cells onto planar patch clamp, for example, e.g., Aviva Bioscience technique. The cell is sealed on-chip and analyzed or broken, and intracellular materials extracted and analyzed; if the cell is not analyzed, cellular material may be positioned for analysis by diffusion, other natural technique, or through micro-fluidic manipulation.

Optionally positioning chip 101 comprises microelectronic array or microfluidic assay, including electrodes or biosensors in which at least one microelectrode or sensor

cavity or element is capable of generating controllable electric current or voltage for drawing probes, samples, or reagents to locations on sensor platform 102, allowing faster, controlled hybridization or analysis.

Positioning chip 101 may use micro or nano-chips with nanoscale channels or 5 membranes, e.g., iMEDD NanoPORE membranes. Depending on size of such membranes, pores selectively exclude antibodies or proteins, while allowing free exchange of glucose, nutrients, insulin, or other molecules. Positioning chip 101 may position mammalian cells of host organism, as well as bacterial, fungal, protozoan, or other unicellular or multi-cellular organisms for analysis.

10 Additionally positioning chip 101 may detect or collect micro-metastatic tumor cells circulating in the blood stream or other body fluids, including but not limited to nipple aspirate, cerebrospinal fluid, peritoneal wash, sputum or excrement such as urine and stool. Preferably enrichment of tumor cells from blood stream may occur in miniaturized or microelectromechanical (MEMs) version of device such as autoMACS to 15 collect circulating carcinoma cells from blood of patients with urologic cancers, or similarly using nanoparticles conjugated with antibody to Epithelial Cell Adhesion Molecule to enrich for circulating tumor cells (CTC) of epithelial origin.

Further using positioning chip 101 in detection or collection, circulating prostate cancer cells in peripheral blood may be enriched, e.g., using technique by OncoQuick in 20 Greiner, Germany, by using anti-human epithelial antigen paramagnetic microbeads or enrichment for disseminated breast cancer cells using advanced density gradient centrifugation; circulating endothelial cells serve as marker for vessel formation and

vascular damage in cancer patients, such circulating cells being detectable for collection from peripheral blood using immunomagnetic beads coupled to antiCD146, an antibody raised against human umbilical vein endothelial cells.

Preferably collected tumor cells are analyzed on biosensor platform 102; for example, disseminated breast tumor cells may be analyzed by multiplex real-time RT-PCR (reverse transcriptase polymerase chain reaction) for mammoglobin, gabaII, B305D-C and B726P, or polymorphisms in carcinogen detoxifying UDP-glucuronosyl transferase UGT1A7 in blood of patients with cancer of proximal digestive tract. Also enriched, using anti-epithelial cells antibody Ber-EP4, e.g., Dynal Corporation technique, epithelial cells derived from peripheral blood of prostate cancer patients can be analyzed using nested RT-PCR-PSA (reverse transcriptase polymerase chain reaction prostate specific antigen) assay as sensor mechanism.

Biosensor platform 102 may employ twenty-five epithelial tumor cells in bone marrow and lymph nodes of esophageal carcinoma (pT1-3, pN0-1 and pM0) patients collected, using cytokeratin and EpCAM antibodies, respectively, by positioning chip 101 for micromanipulation in biosensor platform 102. Further DNA amplified by DNA sensor 201 using Mse-adapter PCR method may be analyzed by comparative genomic hybridization (CGH) for DNA-gains, -losses and point mutations by single-strand conformation polymorphism (SSCP). Also total RNA isolated PBMC in peripheral blood of breast cancer patients, may be subject to RT-PCR luminometric hybridization assay for presence of human telomerase reverse transcriptase, which is highly expressed in majority of tumor cells.

During sensing operation, positioning chip 101 may place samples on biosensor platform 102 for analysis. Biosensor platform 102 measures, detects, sequences, and other biological activities in serial or parallel in or out of organism. Biosensor platform 102 may use multi-functional high-throughput and density biochip having micro or 5 nanoarrays, having substrates manufactured using glass, nylon, silicon, ceramic, metal, gel, membranes, synthesized nanomaterials, or other material.

Biosensor platform 102 provides data gathered from sensor arrays to network 103, which provides data to systems-biology platform 104, where data is integrated or processed. Systems-biology platform 104 may analyze empirically-sensed and simulated 10 factors of individual organism in combination, to determine or confirm host profile of personal biological processes or makeup.

Systems-biology platform 104 may convey processed information to network 103. Network 103 communicates processed data to components coupled to network 103, including data storage 105, wireless communication unit 106, therapeutic manufacture 15 108, therapeutic reservoirs 109, or sensor manufacture 110.

Data storage 105 keeps records or stores processed data by systems-biology platform 104. Processed data from systems-biology platform 104, through network 103, optionally may be conveyed to wireless communication unit 106. Wireless communication unit 106 provides processed data access to external source, such as 20 Global Positioning Satellite (GPS) receiver unit, media repository, personal computer (PC) or workstation, laptop, handheld computing device, cellular device, internal or external camera, another internal implantable or attached sensor or chip, external

biological monitoring device, outside network, healthcare provider, pharmacist, insurance agent, or other device or service communicating with bio-sensor.

5 Processed data from systems-biology platform 104, through network 103, may be conveyed to therapeutic manufacture 108, where therapies are manufactured according to host biological status or simulation output. Effectiveness or side-effects of therapies, produced by therapeutic manufacture 108, are monitored by biosensor platform 102. Ongoing or intermittent feedback from biosensor platform 102, through network 103, to therapeutic manufacture 108 provides automated or iterative therapeutic process.

10 Optionally therapeutic manufacture 108 stores biological therapies in therapeutic reservoirs 109. Therapeutic manufacture 108 or therapeutic reservoirs 109 communicate through network 103 for filling or dispensing. Processed data from systems-biology platform 104, through network 103, may be conveyed to therapeutic reservoirs 109, where respective therapies are released according to biological status. Effectiveness or side effects of therapies, released by therapeutic reservoirs 109, are monitored by 15 biosensor platform 102. For example, biosensor platform 102 may sense therapeutic effectiveness or side effects, while systems-biology platform 104 analyzes negative or positive effects to make recommendations. Ongoing feedback from biosensor platform 102, through network 103, to therapeutic reservoirs 109 provides automated or iterative therapeutic cycle.

20 Processed data from systems-biology platform 104, through network 103, optionally is conveyed to sensor manufacture 110. Sensor manufacture 110 comprises hardware or software-programmable (reconfigurable and software-programmable terms

may be used interchangeably) biosensors *in vivo* that integrate into biosensor platform 102 for supplementary sensing. Sensor manufacture 110 may be used to monitor additional biological materials originally part of biosensor platform 102, as well as used functionally to replace damaged sensors. Sensor manufacture 110 may be used to sense 5 newly-calculated operational conditions by systems-biology platform 104. Optionally sensor manufacture 110 may monitor interactions between novel drug therapies, produced by therapeutic manufacture 108, and organism biology.

Appropriate timing of functions is preprogrammed before biosensor 100 is attached or implanted into organism. Time intervals for sensing are programmed 10 according to external diagnosis, which can range from seconds, minutes, hours, weeks, or longer. Once initial sensing begins, timing adjusts based on processed information by systems-biology platform 104. For example if genetic mutations within genome are found to be rare within multiplying cells, systems-biology platform 104 instructs biosensor platform 102 not to monitor genome as frequently.

15 Conversely if sensed or simulation parameter, input vector, stimulus, condition, environment or other host biological factor is changing frequently, or there is a high risk of change, then systems-biology platform 104 instructs biosensor platform 102 to increase frequency of particular sensor or assay. For example if organism changes through organ transplant, or is infected with new virus, systems-biology platform 104 20 instructs biosensor platform 102 to increase the monitor frequency of antigen or antibody responses while decreasing such factors that are relatively stable.

Figure 2 shows biosensor platform 102 with multifunctional array 200 coupled to detection system 230, and integrated sensor and detection system 231. Multifunctional array 200 serves as programmable or logical interconnect for coupling or switching various sensor devices, and interacts with samples and detection system 230 interprets 5 samples into data to be analyzed by systems-biology platform 104. Multifunctional array 200 may include micro and nanoarrays (M/N arrays) and biochips to test or monitor biological functions in particular organism.

Sensor components may include deoxyribonucleic acid (DNA) sensor 201, ribonucleic acid (RNA) sensor 202, peptide or protein sensor 203, antibody sensor 204, 10 antigen sensor 205, tissue factor sensor 206, vector and virus vector sensor 207, lipid and fatty acid sensor 208, steroid sensor 209, neurotransmitter sensor 210, inorganic ion and electrochemical sensor 211, pH sensor 212, free radical sensor 213, carbohydrate sensor 214, neural sensor 215, chemical sensor 216, small molecule sensor 217, exon sensor 218, metabolite sensor 219, intermediates sensor 220, chromosome sensor 221, or cell 15 sensor 222. M/N arrays are arranged architecturally as micro-electromechanical system (MEM) or as nano-electromechanical system (NEMS). This miniaturized architecture, as MEMS or NEMS device, allows multiple M/N arrays in a condensed form.

DNA sensor 201 is used to detect presence and/or sequence and/or structure of any DNA molecules including profiling for changes in methylation, monitor gene 20 expression, undergo gene and DNA mapping, library screening, functional screen assays for nonsense and frame-shift mutations, scan the whole genome including micro-array-based comparative genomic hybridization to measure and map DNA copy number aberrations, detect disease markers, genotype single nucleotide polymorphisms (SNPs)

including loss of heterozygosity analysis using SNP array hybridization and single-strand conformation polymorphism (SSCP), genotype organisms, examine protein-DNA interactions, and determine genetic characteristics individual to the organism.

DNA sensor 201 utilizes high-throughput M/N arrays for hybridization and use
5 biochips, such as oligonucleotide M/N arrays, antibody M/N arrays, P1-based artificial chromosome (PAC) M/N arrays, bacterial artificial chromosome (BAC) M/N arrays, yeast artificial chromosome (YAC) M/N arrays, cosmid M/N arrays, cDNA M/N arrays, gene M/N arrays, whole-genome M/N arrays, SNP M/N arrays, gridded cDNA M/N arrays, Southern Blots, theme M/N arrays (array centered around a particular disease or
10 gene family), bead M/N arrays (arrays made up of small beads containing capture oligonucleotides), bead based M/N arrays (arrays in which reactions take place on the surface of microbeads), gel-pad M/N arrays (arrays in which chemical and enzymatic reactions can be carried out on three dimensional pads, like miniature test tubes), microcantilever arrays (in which specific biomolecular interactions occur on one surface
15 of a cantilever beam, such as changes in intermolecular interactions that generate sufficient surface stress to bend beam for optical detection, M/N gel electrophoresis chips and M/N arrays 2D gel electrophoresis chips, chromatographic protein M/N arrays, e.g., Ciphergen protein sensor, and hybridization techniques for deoxyribonucleic acid sensing. Phenotypic markers for DNA damage or repair include single-cell gel
20 electrophoresis use comet assay in which DNA damage is visualized, e.g., Komet 4.0 by (Kinetic Imaging Ltd) Imaging System.

Optionally for single nucleotide polymorphism (SNP) detection, DNA sensor 201 may apply so-called invader platform, or other device for genetic sequencing of an

individual. DNA sensor 201 can analyze peritoneal fluid from patients with ovarian cancer for loss of heterozygosity (LOH) at chromosomal arms 13 q (BRCA2 locus), 17 (BRCA1 and p53 loci) and 22q and for mutations in their p53 and k-ras genes. It can detect SNP (936 C>T) in 3' UTR of vascular endothelial growth factor gene (VEGF) in

5 DNA extracted from blood of patients with breast cancer.

Further DNA sensor 201 can identify polymorphisms in carcinogen detoxifying UDP-glucuronosyl transferase UGT1A7 in blood of patients with cancer of the proximal digestive tract. Also methylation abnormalities in the promoter CpG islands of p16, HOX A9, MAGE A1 and MAGE B2 can be detected in sputum of lung cancer patients

10 with DNA sensor 201. Sharply-elevated levels of stool DNA can be detected by DNA sensor 201 in patients with colorectal cancer. Stool DNA of surface epithelial cells is quantified using Picogreen fluorimetry.

DNA sensor 201 can detect chromosomal aneuploidy in cervical intraepithelial neoplasia or dysplasia using interphase cytogenetic technique called dual-color

15 fluorescence in situ hybridization (FISH) targeting chromosomes 1, 7, 9 and 17 in Pap-smear slides and a thin layer of cervical cells.

Using DNA sensor 201, nipple aspirate fluid (NAF) containing epithelial cells shed from the breast ductal system can be analyzed. Extracted NAF DNA can be PCR amplified and analyzed for loss of heterozygosity in nuclear genome and deletions in

20 mitochondrial genome using microsatellite markers and primer pairs, respectively.

Further DNA sensor 201 can be used to detect acute lymphoblastic leukemia prenatally by analyzing fetus blood to detect TEL-AML1 by FISH and genomic

breakpoints by long-distance PCR. Using DNA sensor 201 and genomic DNA from whole blood, germ line polymorphism in KLK10 at codon 50 (GCC to TCC) associated with risk of occurrence in prostate cancer can be detected.

Also using DNA sensor 201, epigenetic changes, such as changes in GSTP1 5 methylation associated with prostate cancer can be detected in bodily fluids, e.g., urine and plasma, of prostate cancer patients. This detection uses real-time quantitative MSP and conventional MSP.

Further DNA sensor 201 is used to search for pieces of DNA in blood that are abnormally long, which is a signature of dying cancer cells; this test can be used for early 10 diagnosis for patients with gynecologic and breast cancers. Optionally oligonucleotide array-based genotyping platform, such as Perlegen, is used for accelerated SNP analysis, allowing whole-genome scanning by DNA sensor 201.

RNA sensor 202 may be used to detect presence, sequence or structure of RNA molecules, such as spliced and un-spliced RNA, mRNA, tRNA, rRNA, improperly 15 transcribed RNA, properly transcribed RNA from diseased DNA sources, ribozymes, RNAi mechanism and application in relation to cancer therapy, or changes or differences in mRNA levels, or structures made of ribonucleic acids. RNA sensor 202 utilizes high-throughput M/N arrays for hybridization techniques, inclusive of DNA sensor 201. Probes may be made to hybridize with RNA molecules, and Northern blot may be used in 20 place of Southern blot technique.

RNA from enriched epithelial cells using anti-epithelial cells antibody Ber-EP4, e.g., per technique by Dynal Corporation, derived from peripheral blood of prostate

cancer patients is analyzed for using nested RT-PCR-PSA assay by RNA sensor 202. Further, RNA sensor 202 can be used instead of second-look laparotomy in women with ovarian carcinoma treated with surgery and chemotherapy and show no sign of disease. Processed peritoneal washings are analyzed by telomerase repeat amplification protocol 5 (TRAP) assay to detect residual disease. Total RNA isolated PBMC in peripheral blood of breast cancer patients, subjected to RT-PCR luminometric hybridization assay for presence of human telomerase reverse transcriptase that is highly expressed in majority of tumor cells.

Peptide or protein sensor 203 is used to detect primary, secondary, tertiary, or 10 quaternary structures or activity of amino acid-based structures, such as sequence, enzymatic activity, protein function, interactions with agonists and antagonists, interactions with organic or inorganic structures or molecules, interactions with membranes, folding and enzymatic changes resulting in external factor, such as temperature, pH, ion concentrations, etc., N or C terminal characteristics, prions and 15 misfolded proteins, amount and concentrations of proteins, bound and unbound state of proteins, sub-cellular localization, phosphorylated and dephosphorylated states, stages of degradation by proteases, stages of translation, gene and protein expression levels, e.g., using techniques such as ANTIBIOMIX (Milagen, Inc.) or Antigen Retrieval (Biogenex Laboratories, Inc.), protein-protein interactions, protein-small molecule interactions, 20 protein-antibody interactions, protein mutations due to transcription and translation mistakes, or measurable factors associated with amino acid based structures. Sensor 203 may be implemented using electrophoresis tag or microassay to identify protein or gene simultaneously, e.g., Aclara eTag assay (Mountain View, CA).

Peptide or protein sensor 203 utilizes high-throughput M/N arrays for hybridization and use biochips, such as protein M/N arrays, proteome M/N arrays, whole-proteome M/N arrays, electrospray fabricated protein M/N arrays, gene expression M/N arrays, reverse transfection M/N arrays (for example membrane proteins that are difficult to purify), functional protein M/N arrays, Western blotting, microcantilever arrays, or quantitative and qualitative high-throughput techniques for amino acid entities.

Peptide or protein sensor 203 can be used to detect proteins in cerebrospinal fluid of patients with primary brain tumors. Differentially-expressed proteins in processed CSF are digested and peptides identified by mass spectrometry. Presence of tumor-related proteins such as VEGF and VAV signifies presence of a primary brain tumor (179). Sensor 203, like SELDI protein-chip, similarly may be used to identify sixteen protein biomarkers in urine of bladder cancer patients, or instead of second look laparotomy in women with ovarian carcinoma who have been treated with surgery and chemotherapy and show no signs of disease. Processed peritoneal washings may be analyzed for telomerase activity to detect for residual disease.

Protein or peptide sensor 203 may be used in detection of diminished levels of N-CAM of < 130 kDa in human serum of patients with brain tumors and the 80 kDa form associated with glioma. Further, protein and peptide sensor can be used in diagnosis of breast cancer by analysis of nipple aspirate fluid (NAF). Using SELDI-TOF capability, the presence of peptides at 4233.0 Da and 9470.0 Da is associated with cancer and the presence of 3415.6 Da and 4149.7 Da may be expected for normal samples. Thus sensor 203 can differentiate between diseased and unaffected populations.

Similarly protein sensor 203 may be used in breast-cancer diagnosis by analysis of serum samples. Samples applied to metal affinity capture chips activated with Ni^{2+} . Using SELDI protein chips/ mass spectrometry feature and software to detect selected discriminatory peaks separate cancer from non-cancer groups.

5 Using same features of sensor 203, serum is analyzed to differentiate between hepatocellular carcinoma (HCC) and chronic liver disease (CLD), where α -fetoprotein fails as biomarker. Detecting 151 potential biomarkers in this way, system can provide diagnosis method for HCC. Using protein sensor 203 in diagnosis of prostate cancer, protein of 50.8 kDa can be detected in serum even where PSA levels are < 4ng/mL.

10 Further protein sensor 203 may be used in diagnosis of colorectal cancer detecting elevated HER-2 levels using standard ELISA and immuno-histo-chemistry (IHC) techniques. Elevated levels of secreted urokinase-type plasminogen activator (uPA) can be detected by sensor 203 in serum for diagnosis of pancreatic cancer using sandwich ELISA or similarly, elevated levels of kallikrein 10 in serum for diagnosis of ovarian 15 cancer, or elevated levels of basic fibroblast growth factor (bFGF) in nipple aspirate fluid in diagnosis of breast cancer, or elevated levels of fibroblast growth factor-2 and pleiotropin in serum for testicular cancer diagnosis or interleukin 6 in the serum of hormone-refractory breast cancer patients using immunoassay.

20 Antibody sensor 204 may be used to detect monoclonal or polyclonal antibodies. Similar to above sensors, hybridization with M/N arrays may be used. Probes may be chemical or molecular biological material that hybridize to targeted antibody, such as

DNA, RNA, peptide, protein, small molecule, steroid, or lipid. Microcantilever arrays and other binding techniques can be applied.

Antibody sensor 204 may use so-called phagotope biochip to display phage with epitopes that react with antibodies in sera of patients with ovarian cancer, or other cancers. Also presence of elevated levels of anti-survivine autoantibody in serum of head or neck cancer patients is detected by antibody sensor 204 using recombinant protein survivine as antigen.

Antigen sensor 205 may be used to detect or recognize individual immune response factors. For example antigen sensing may detect autoimmune response factors, such as sensing multiplex character autoantibody response in systemic lupus erythematosus, rheumatoid arthritis, or multiple sclerosis. Another example of antigen sensor 205 application may be identification or targeting of cell surface antigens for cancer therapy, e.g., Genentech approach.

Antigen sensor 205 may be used for early diagnosis of lung cancer or efficacy of chemotherapy by detecting nucleosomes in serum using assay, e.g., Cell Death Detection ELISAPlus (Roche Diagnostics). Further antigen sensor 205 may detect tumor-associated antigens such as CYFRA21-1 for non-small cell lung cancer, and CEA, NSE and ProGRP for small-cell lung cancer.

Other sensing techniques for cancer detection contemplated herein include antimalignin antibody screen test and tests for cancer markers including alpha fetoprotein (AFP), CA 15.3, CA 19.9, CA125, carcinoembryonic antigen (CEA), EVP test for epstein bar virus, T/Tn Antigen test, TK-1 test and prostate specific antigen (PSA) or free

PSA (fPSA) test. For bladder-cancer bladder-tumor-associated antigen test (BTA), BTA stat test, BTA TRAK test, fibrin/fibrinogen degradation products test (FDP), and NMP22 assay. Protein-based markers may illuminate and map abnormal cells, e.g., Inpath system. Other blood tests include CBC blood test, biological terrain assessment (BTA),

5 Pre-Gen 26, telomerase test or DR-70 test.

Tissue-factor sensor 206 may use tissue factor M/N array to sense tissues, tissue factors, or tissue origin, using probes or antibodies to hybridize with targets. Tissue-factor sensor 206 may detect increase in prostaglandin E₂ production in cells that over-express COX2. This detection is associated with enhanced growth, migration and

10 invasion as in bladder tumors.

Lipid or fatty acid sensor 208 may provide membrane mapping, M/N gel electrophoresis chips and M/N arrays 2D gel electrophoresis chips, detergent analysis, M/N array analysis of glycolipids and membrane proteins, membrane fluidity analysis, cholesterol analysis, or other test to examine cellular or intracellular organelles lipid

15 bilayers.

Lipid or fatty acid sensor 208 may detect changes in exposed membrane; for example, such sensor 208 may produce antibody, with traceable label conjugated thereto, to anionic phospholipids (AP), such as phosphatidylserine, phosphatidylinositol and phosphatidic acid, that are more specific for AP than annexin V. When released into

20 blood stream this antibody binds activated, by inflammatory cytokines, hypoxia, hydrogen peroxide, thrombin or acidic conditions, endothelial cells and thus, tumor blood

vessels have increased exposure of anionic phospholipids on their surface. Localization of label enables localization of tumor.

Lipid or fatty acid sensor 208 may detect levels of accumulation of synthetic membrane-permeable alkyl-lysophospholipids (ALPs), such as Edelfosine, Mitelfosine and Perifosine, that are anticancer agents that interfere with lipid mediated signal transduction.

Vector or virus vector sensor 207 may use microarray or assay with known sequenced virus attached, e.g., DeRisi Laboratory. Unknown viruses may be detected through examining homology to known viruses, and subsequent arrays can be manufactured by sensor manufacture 110 to detect new viruses. Optionally assays that detect homologs can be applied, such as Celera Diagnostic Viroseq™ HIV system for detection of mutations in human immunodeficiency virus (HIV) genome that confer drug resistance. Optionally assays for virus RNA can be used, such as Bayer Diagnostic Versant® HIV-1 RNA 3.0 Assay for qualification of HIV-1 RNA in plasma of infected people.

Further microparticle enzyme immunoassay (AxSYM HbsAg V2), e.g., Abbott Laboratories, may be used in quantifying reactivation of HBV during chemotherapy for lymphoma with Doxorubicin along with real-time quantitative PCR specific to region of major S protein. Virus and virus vector sensor 207 may be used for detection of oncolytic virus replication in tumor tissues.

Steroid sensor 209 detects levels of steroids in the body, and monitors or controls levels of steroid hormones. Sensor 209 targets hormonal changes associated with puberty, menopause as well as fitness-conscious steroid-pumping athletic types.

Neurotransmitter sensor 210, small molecule sensor 217, or exons sensor 218
5 detects using M/N arrays, such specific antibodies as probes that hybridize with desired targets. Inorganic ion or electrochemical sensor 211 may detect ionic concentrations using techniques, using MEMS technologies with dielectric currents, microfluidics, or dialysis on a N/M platform. pH sensor 212 may read pH by detecting H_3O^+ concentrations like silicon oxide pH sensors, e.g., Intelligent Pill. Free radical sensor
10 213 may be used to measure free radical activity, by using antioxidants as probes.

Carbohydrate sensor 214 may use oligosaccharide arrays, polysaccharide arrays, or carbohydrate chips, e.g., Glycominds glycochip, to measure glycan-protein interactions such as enzymes, antibodies, and lectins. Branched carbohydrates may bind to lectins involved in cell adhesion and migration processes. Also, natural branched
15 carbohydrate like Lewis y, which is over-expressed in, for example, colon and ovarian cancer may be detected by carbohydrate sensor 214. Such sensor 214 may apply to whole blood glucose (WBG) monitoring system, or continuous glucose monitor, e.g., Sensors for Medicine Science.

Neural sensor 215 measures action potentials or voltage between neurons in
20 central nervous system, using thin-film M/N electrodes as front-end sensors in MEMS and NEMS.

Chemical sensor 216 senses native or foreign chemicals, such as toxins, pharmaceuticals, vitamins, minerals, or other organic or inorganic chemicals. Chemical M/N arrays may be used, in which arrays of small organic compounds may be used to analyze interactions of proteins with various compounds. Conversely proteins or RNA 5 may be used as probes to detect chemical substances.

Chemical sensor 216 may measure levels of carcinogen, benzo(*a*)pyrene diol epoxide, a metabolic product of benzo(*a*)pyrene found in tobacco smoke, known to cause 9p21 aberrations in peripheral blood lymphocytes in bladder cancer cases. Further chemical sensor 216 may measure tobacco-specific carcinogen 4-(methylnitrosamino)-1- 10 (3-pyridyl)-1-butanone (NNK) that can induce transformation of human breast epithelial cells, and may be directly related to initiation of human breast cancer in smokers.

Metabolites sensor 219 uses protein or antibody M/N arrays that hybridize to particular metabolites. Sensor 219 is useful to detect excessive buildup of metabolites. For example metabolites sensor 219 can measure serum homocysteine levels, associated 15 with increased risk of cervical cancer, and further DNA sensor 201 may detect common polymorphisms in one-carbon metabolic pathway; examples of such mutations include MTHFR C677T, MTHFR A1298C and MTR A2756G. Increasing copies of MTHFR 677 variant polymorphism is associated with increased homocysteine levels whereas increasing copies of MTR 2756 variant polymorphism is associated with decreased levels 20 of such metabolite.

Intermediates sensor 220 uses various protein and antibody M/N arrays that hybridize to particular intermediates. Sensor 220 is useful to detect excessive buildup of

intermediates; also sensing specific sequence, tertiary or quaternary structure of intermediates is used in drug design specificity.

Chromosome sensor 221 senses abnormalities in folding of chromosome, such as faulty histones, senescence-associated heterochromatic foci, or SAHF, since genes 5 contained in these chromosomal regions are switched-on in proliferating cells, but are switched-off or "silenced" during cellular senescence.

Cell sensor 222 attaches whole living cells as probes, and is used for interactions with whole cells, such as cytotoxicity, drug metabolism, pharmacokinetics, target validation, interactions with other cells, extracellular materials, phenotypic analysis of 10 genes and interfering RNA, as well as other biomolecules and compounds, e.g., Excellin Life Science bionic chip, which provides cell growth on chip. Effectively the cell becomes part of the chip, which allows manipulation and analysis of cell using microelectronics; the chip sends electrical signals through an on-board living cell, which detects changes in cell-membrane structure. The bionic chip can monitor and detect 15 conditions that can cause cellular damage.

Optionally image cytometric measurement of breast fine needle aspirates can be used in cell sensing to predict nodal involvement in breast cancer. DNA ploidy, S-phase fraction, G0G1/G2M ratio, and minimum (start) and maximum (end) nuclear pleomorphism indices (NPI). Further cytometric imaging allows differentiation between 20 normal cells in which PML protein resides in discrete PML bodies and promyelocytic leukemic cells in which PML protein is genetically rearranged or dispersed throughout the nucleus.

Sensor unit 111 may measure or transmit blood pressure, flow rate or other sensor data wirelessly to controller unit 112, similarly to so-called cardioMEMS devices for monitoring pressure within aortic aneurysm. Biosensor 100 is implanted using catheter and transmits data to controller unit 112. Optionally such device can be used assessing 5 circulation to organ after transplant or reconstructive surgery. This provides physician with early indication of whether surgery is successful and prevent irreversible damage to organ.

Biosensor 100 may use implantable blood-flow monitoring system for providing synchronized blood vessel flow or myocardial wall contractility data to external monitor 10 independent of transcutaneous leads. Further, since heart failure (HF) status of a patient is determined based on morphology of signal representative of arterial pulse pressure, the signal can be plethysmography signal that is produced by implantable or non-implanted sensor.

Time-derivative sensed signal may be produced based on signal representative of 15 arterial pulse pressure; time derivation signal can be used to determine maximum and minimum peaks of signal representative of arterial pulse pressure. HF status can be assessed directly from time-derivative signal.

Biosensor 100 can be implanted using placement catheter, endoscope, or 20 laparoscope; such device can be secured in LV or heart wall, e.g., using corkscrew, helical anchor, harpoon, threaded member, hook, barb, fastener, suture, or mesh or coating for receiving fibrous tissue growth.

Biosensor 100 provides less-invasive chronic measurement of left ventricular blood pressure or other parameters. Biosensor 100 can perform cardiosaver function to indicate to human subject that myocardial infarction is occurring; data is transmitted wirelessly to controller unit 112 for systems-biology analysis. Therapeutic reservoir 109
5 can inject thrombolytic or anti-thrombogenic agent into bloodstream promptly to dissolve thrombus that caused myocardial infarction, and prevent formation of additional thrombi.

Biosensor 100 may sense impedance measurements of heart, respiratory or patient motion, and from these measurements, generating alarm signal when measurements
10 indicate occurrence of cardiac arrhythmia. Optionally rate-responsive pacing system includes sensor of minimum oxygen content in right atrium over prescribed time interval, and using such minimum oxygen content as control parameter for adjusting rate of pacemaker.

15 Optionally for sensors in multi-functional array 200, nano-particles that specifically bind to particular molecules can be used to detect sequence, folding, binding, interactions, function, or overall characteristics. Once bound to particular biological molecules, arrangement of distances between nanoparticles results in different observable properties, such as color or pattern.

20 Array 200 may be configured electronically by systems biology platform 104 to couple or interconnect selectively according to simulation or modeling to access actual host condition via one or more biosensor signals. Such sensed signal set may be compared by simulator against model or other software prediction to confirm host or target material health or other problem, as described herein.

Nanoparticle arrangement on biological molecules provide or indicate function, e.g., Northwestern University DNA-Driven Assembly of Biomaterials system. By attaching gold particles to DNA nucleotides, DNA hybridizes with complementary strand and creates specific arrangement of gold particles. That arrangement of nanoparticles 5 gives detectable color or pattern, which can be detected by optical device, and DNA can be sequenced.

Measuring color differences between nano-particle arrangement can also be applied to other biological molecule, e.g., Northwestern University Nanoscale Bioassay for Specific Antibodies. Rather than engineering nanoparticles that attach directly to the 10 biological molecule, nanoparticles can be attached to specific antibodies. Binding of antibodies to targeted protein, DNA sequence, small particle, lipid, chemical, or other biological produces a particular color that is detectable or analyzable.

Also Nanoplex Technologies Nanobarcodes Particles, made of different metals attached to biological molecules for multiplexing bioassays use probes attached to 15 alternating metals on Nanobarcodes to hybridize with biological molecules; then current can be run through Nanobarcodes to determine molecules that bind to probes.

Detection system 230 may produce data from hybridization M/N arrays and other analysis techniques, e.g., fluorescent scanners, laser scanning phosphorimagers, mass spectrometry, fiber optics, atomic force microscopy, parallel surface plasmon resonance 20 imaging (allows direct analysis of binding events without need of reporter systems or tags), conclusive-induced dissociation (CID) mass spectra through electrospray ionization tandem mass spectrometry (ESI-MS) on triple or quadruple or ion trap mass

spectrometers, real-time polymerase chain reaction (PCR), PCR, Fluorescence *in situ* Hybridization (FISH), or charged coupled devices (CCDs).

Integrated sensor or detection system 231 may produce data from samples, without separating detection from hybridization or other technique. Optionally 5 semiconductor-based M/N array can be used, e.g., CombiMatrix matrixArray; such array allows precise, digital control of electrochemical detritylation, including embedded sensor designed in semiconductor substrate, alternatively to conventional fluorescence technology. Hybridization with array sends direct electronic signals for analysis.

Another example of integrated sensor detection system 231 assay, can be 10 GeneFluidics 3D micro-fabricated platform with embedded electrochemical sensor array. This platform conducts molecular analysis of raw DNA or protein samples, e.g., no PCR or immunoassays. Electrochemical detection of samples, such as whole blood, saliva, stomach acids, or other bodily fluids, uses current to measure electron transfer with current signal, associated with hybridized nanomolecules, e.g., ssDNA, hybridizable 15 nanoparticles).

Biosensor 100 generally comprises biological microelectromechanical (bio-MEMs) sensor chip or detection or transducer device that may be implemented or computer-modeled for operation in silicon, silica, glass, polymer or other substrate or instrumentation cavity, beam, surface, channel, encapsulated molecules, membrane, 20 quantum dot or nanocrystal (e.g., CdS, CdSe, CdTe, ZnSe, or other colloidal group II-VI semiconductor), matrix or array for single or multi-channel independent signal detection in two or three dimensions in vitro or in vivo.

For example, sensor 100 may serve as high-throughput and sensitivity bio-physical, pharmaceutical or chemical recognition probe or cartridge for identification and/or characterization of host tissue or serum DNA, RNA, nucleic acids, protein, lipids, 5 carbohydrates, enzymes, aptamers or other biomolecular or signal reporter target or any interaction, mutation, mass or rate thereof. Also such sensor may provide integrated, monolithic, discrete, or distributed, reagent-based or reagentless, microfluidic lab-on-chip microbiology mass spectrometry, flow immunosensor (e.g., FAST monitor for food or water quality), microarray or microassay functions, such as growing virus, bacteria or 10 other eukaryotic or prokaryotic cells in microcells, nucleotide hybridization, polymerase chain reaction, molecular imprinting, chemical synthesis, ligand fishing, phage selection and concentration, multicomplex formation, diffusion limited concentration, or challenging antibiotics for rapid target detection, antibody susceptibility determination, or affinity and kinetic analysis.

15

Biosensor 100 may be implemented in quartz crystal microbalance for detecting or monitoring physical or chemical associated mass change or dissipation rate. Also whole cell or host sensor detection method may sense radioisotope, fluorescence, colorimetric, electrochemical, chemiluminescence, or bioluminescence. Additionally 20 molecular or lipid-layer membrane-based sensor may operate to report change in electrical ionic, e.g., Ion Channel Switch biosensor using alternating current or voltage.

Furthermore encapsulated molecules may employ probes encapsulated by biologically localized embedding (e.g., PEBBLE nanosensors for intracellular chemical sensing, which may be delivered via gene gun, picoinjection, liposomal delivery, or phagocytosis, use matrices of cross-linked polyacrylamide, cross-linked decyl 5 methacrylate, and sol-gel silica) for H^+ , Ca^{2+} , K^+ , Na^+ , Mg^{2+} , Zn^{2+} , Cl^- , NO_2^- , O_2 , NO and glucose detection; optionally encapsulated outer shell may be modified as configurable platform to target selectively specific biological locations or antibodies, such as including or excluding species variously reactive to passing through or filtered by the polymer 10 membrane.

10

Biosensor 100 may recognize protein for antigen-antibody recognition, particularly by localizing or mapping protein residue epitopes. For example sensor contact at epitope-paratope interface functions via crystallographic analysis of one or more poly- or monoclonal or antigen-antibody complex. Also sensor 100 may detect 15 cross-reactive binding with antiprotein antibodies using synthetic peptides as antigenic binding probe for free peptides or peptides adsorbed to solid-phase, conjugated to carrier or attached to synthesis support.

Additionally sensor 100 may detect cross-reactive binding decrease to identify 20 critical residues in peptides via systematic residue replacement, as well as other protein-protein interaction, for example, between protease-inhibitor, antibody-antigen, enzyme-inhibitor, hormone-receptor, or signal transduction or transcriptional complexes. Protein

sensing analyte may include fatty acids, maltose, biotin, Ca^{2+} , Co^{2+} , Zn^{2+} , Cu^{2+} , glucose, glutamine, or other organic serum or tissue material.

Biosensor 100 may immobilize or control orientation of biomolecular target
5 binding or catalytic sites via adsorption, entrapment behind membrane or in polymer or
sol gel, covalent coupling, surface-immobilized polymer, or other capture system. Sensor
orientation control may be accomplished via covalent coupling with attached glycosides,
generation of specifically-located thiol groups, use of antibody-binding proteins,
avidin/streptavidin capture system, or use of tags with engineered antibody fragments.

10

Additionally sensor spatial control of surface immobilization may use soft
lithography for substrate or surface patterning to introduce surface function, deposition
control by physical placement, light-directed immobilization and patterning, or electro-
chemical deposition control, for example, using elastometric polymer poly
15 dimethylsiloxane PDMS.

Also molecular imprinting polymer sensor may employ affinity sensor where
response is produced by accumulation of template on sensor surface, receptor sensor
where response is generated by change in polymer characteristic or induced by template
20 interaction, or enzyme-mimicking sensor where response is generated according to
change in environment induced by molecular imprinting polymer-mediated catalytic
reaction.

Furthermore antibody-based sol-gel sensor may use competitive assay detection, where antibody is encapsulated in gel, sol-gel sensor is immersed in sample containing analyte concentration and known fluorescently labeled analyte solution, excess analyte is washed from gel, and fluorescence emission from remaining bound analyte is measured optically; displacement assay detection, where antibody is encapsulated in gel with pre-bound fluorescently-labeled analyte, and gel is removed from solution and fluorescence emission from undisplaced analyte is measured; and fluorescence quenching detection, where fluorescently labeled antibody is encapsulated in gel, which is immersed in sample, and bound analyte quenches fluorescence from antibody tag.

10

Biosensor 100 may employ optical biosensor or transducer with various assay formats. Direct assay may not use label, and analyte surface binding is measured directly. Sandwich assay secondary antibody binds to surface-bound analyte molecule after analyte binding to sensor surface. Competitive assay enables binding-site competition on sensor surface, and low sensor signal is obtained for high analyte concentration.

15 Optical transducer sensor may use input grating coupler (e.g., bidiffractive grating coupler), prism coupler, planar or nonplanar, polarimetric, ion-exchange or deposited-rib, channelized or non-channelized waveguide or interferometer (e.g. Mach-Zehnder interferometer), as well as surface plasmon resonance sensor (e.g., BIACORE system) using prism coupler, resonant mirror with vibro-stirrer (e.g., lasys), evanescent wave fiber optic biosensor for multi-analyte detection (e.g., RAPTOR antibody identification

system), displacement flow detector, or other optical or time-resolved or phase fluorescence transducer (e.g., to detect fluorophore-labeled binding protein or fluorescence resonance energy transfer), or fiber optic elements.

5 Biosensor 100 may employ acoustic transducer or wave device, such as bulk or surface acoustic wave device, thickness-shear mode resonator, shear-horizontal surface acoustic wave, acoustic plate mode, or love wave sensor, for example, to detect and characterize sensitive biological binding events in real time without labeling, by measuring energy loss occurring at liquid-solid biomolecular interface.

10 Biosensor 100 may employ fast-flow injection or microtiterplate immunoassay using enzymatic amplification electrodes, for example, via bi-enzymatic substrate recycling for signal amplification using electrochemical or bioelectrocatalytic redoxlabel immunoassay. Bioelectrocatalytic sensor electrode material for detecting phenolic targets
15 via alkaline phosphatase measurement, for example, may include glassy carbon, graphite, carbon paste or ink, or gold.

20 Preferably sensing devices or techniques are provided or performed in miniaturized implantable format. However some sensor devices or methods may require sample from implanted device to be transferred to instrument located outside the body. Data generated by such instrument is transmitted to systems-biology platform 104 for analysis or modeling.

Biosensor platform 102 sensors, detection systems, or components may apply to parasitic or symbiotic organisms, such as bacteria, fungi, protozoa, plant, or other unicellular or multi-cellular organisms provided in host organism. For example DNA sensor 201 may sense DNA structure of fungus cell living within such organism, peptide or protein sensor 202 may read its protein structures, and other sensors may read other biological properties. This information along with data from host organism is interpreted with systems-biology platform 104, and solution to expunge fungi is calculated or implemented.

10 Figure 3a shows software components of systems-biology platform 104. Once biosensor platform 102 produces comprehensive data on system, it is sent to network 103 and processed or analyzed by systems-biology platform 104.

15 Systems-biology platform 104 analyzes overall or partial structure of system or host, combining data from sensor components as well as model data of biosensor platform 102. Systems-biology platform 104 uses software for analyzing genomics 301, proteomics 302, computational chemistry 303, pharmacogenomics 304, computational biology 305, computational biophysics 306, computational cell behavior 307, pharmacokinetics 308, metabolomics 309, transcriptomics 310, bioinformatics 311, other 20 computational behavior of the biological system, or other “omics” studies.

Other software may be integrated to understand or implement biological system on personalized level, e.g., specific gene sequence, individual protein interactions, personal localized mRNA levels, dynamics of particular system, methods of control, personal cytotoxicity, and methods to design and modify the system; comprehensive data set is generated to understand fully or partially subject organism.

Genomics 301 may map, sequence, analyze, or discover function of organism genome. Structural or functional genomics may be used in genomics 301. Proteomics 302 analyzes organism proteome, describing set of proteins expressed during lifetime of cell or group of cells. Proteomics 302 calculates structure determination, at lower level, to functional analysis, or cell modeling at higher level of modeling.

Computational chemistry 303 uses algorithmic tools to facilitate chemical analyses. Chemical analysis occurs at atomic or molecular level, examining how individual and groups of atoms, compounds, or other structures interact with living system; further it analyzes chemical relationships between biological structures.

15 Pharmacogenomics 304 calculates potential drug responses based on personalized genetic information. This information is useful for determining appropriate therapies or preventing adverse reactions.

Computational biology 305 uses algorithmic tools to facilitate biological analyses. Computational biophysics 306 uses algorithmic tools to facilitate biophysical or 20 biokinetic analyses. Computational cell behavior 307 uses algorithmic tools to facilitate complete analyses of intracellular or intercellular behavior.

Pharmacokinetics 308 determines or predicts kinetic interactions between potential drugs and organism biological molecules, taking into account variable interaction factors, such as sterics, charge, dipole forces, or other factors that determine molecular interactions.

5 Metabolomics 309 analyzes organism overall metabolic profile, such as metabolism rates, amounts of metabolite intermediates, metabolic efficiency, structure of metabolic proteins, interactions between metabolic proteins and therapies, phosphorylative rates, or other aspects of individual metabolism.

10 Transcriptomics 310 analyzes organism transcription profile, such as efficiency, transcription errors to mRNA, intron-exon-splicing, biological transcription machinery, or other attributes of organism transcription.

15 Bioinformatics 311 undergoes database-management activities, involving persistent sets of data that are maintained in consistent state over indefinite periods of time. Bioinformatics 311 provides information content or flow in biological systems and processes; it serves as bridge between observations (i.e., data) in diverse biologically-related disciplines and derivations of understanding (i.e., information) about how systems or processes function, or subsequently the application.

20 Figure 3b shows ability to transfer information between systems-biology platform 104 and data storage 105 through network 103. This allows comparative studies between previously programmed and stored data with real-time computation; comparative studies serve as check against errors made by biosensor platform 102, and provide insights into overall systems understanding.

Also data storage 105 stores information processed by systems-biology platform 104. Data storage 105 may be located internally or externally relative to the organism, which can be accessed through wireless communication unit 106.

Regulation software or overlay 320 couples to data storage 105. When systems-
5 biology platform 104 communicates with data storage 105, regulation overlay 320 assures that therapies, instructions, or other communications complies with Food and Drug Administration (FDA), Patent and Trademark Office (PTO), or other government regulatory bodies.

Regulation overlay 320 can store information or instructions for private
10 agreements or regulations, such as contract or licensing agreement between biosensor 100 and pharmaceutical company. Depending on severity of organism condition or systems-biology platform 104 suggested therapy, communication directly or indirectly with FDA may be possible in instances where “expanded access,” “compassionate use,” “well characterized biological products,” and other FDA exceptions apply. FDA may respond
15 favorably and allow use of unapproved therapy (suggested by systems-biology platform 104) if exceptions apply.

Systems-biology platform 104 may implement neural network to model biological system or serve as decision aid for medical applications, problems or diagnosis. For example such platform 104 may employ methods as pattern recognition, feature extraction, supervised learning, unsupervised learning, or learning algorithms.
20 Supervised learning methods may include Fisher’s Linear Discriminant, Gradient Descent Procedures, Perceptron Algorithm, Relaxation Procedures, or Potential

Functions for linearly separable sets, or Nonlinear Discriminant Functions, Hypernet, Minimum Squared Error Procedures (MSE), or Ho-Kashyap Procedure for nonlinearly separable sets.

For multiple category classification problems, supervised learning methods may 5 include the Fisher Linear Discriminant, Kesler Construction, or Backpropagation.

Unsupervised learning methods may include clustering, Kohonen networks, Kohonen Competitive Learning, Hebbian learning, Adaptive Resonance Theory (ART) or prototype distribution map (PDM). Clustering approaches may include Basic Isodata Procedure, similarity measure approach, or criterion functions.

10 Criterion functions approaches may further include sum of squared error criteria, minimum error criteria, or scattering criteria, and such criteria may be used in an iterative optimization procedure. Platform 104 may also employ clustering approaches such as hierarchical clustering or metrics.

To assist in medical decision-making, systems-biology platform 104 may 15 implement artificial intelligence or decision techniques, particularly data-based techniques or knowledge-based techniques. Data-base techniques may include approaches such as database, decision theory, pattern recognition, or Bayesian analysis, while knowledge-based techniques may include mathematical modeling and simulation, symbolic reasoning, as well as databases.

20 Systems-biology platform 104 may employ database such as patient record structures (e.g., hierarchical databases, National Library of Medicine, MUMPS (Massachusetts General Hospital Utility Multi-Programming System), ARAMIS system,

PROMIS (problem-oriented medical information system), or medical database management system (e.g. MEDUS/A)). Systems platform 104 may employ disease database (e.g. oncology, rheumatology), or decision-support system (e.g. HELP program).

5 Platform 104 may employ differential diagnosis database (e.g. RECONSIDER or DXplain), online database, radiological database (e.g. CHORUS (collaborative Hypertext of Radiology)), or Human Genome Project. Mathematical modeling and simulation may apply to modeling of organism or biological process. Biological process may be represented by mathematical equations and evaluated.

10 Simulation involves representation of organism or biological process on a computer. Mathematical formulation may apply to administration of drugs or analysis of drug toxicity or drug level in a biological system. Pattern-recognition techniques may include discriminant analysis, method of classification using Bayes' Rule, parameter estimation, supervised learning, or unsupervised learning.

15 Unsupervised techniques may include Parzen windworks, k-nearest neighbor estimation or other learning clustering techniques. Decision theory techniques may employ Bayesian analysis or Markovian analysis. Symbolic reasoning techniques may employ knowledge-based expert systems including early expert systems, second-generation expert systems. Techniques of expert systems may include knowledge representation, heuristic search, natural language understanding, and exact reasoning. Second-generation expert systems may employ causal models, reasoning with uncertainty, or hybrid systems.

Systems-biology platform 104 may implement fuzzy techniques, (e.g. fuzzy set theory, fuzzy logic, fuzzy variables, or membership functions) for use in neural networks and expert systems. In dealing with uncertainty in supervised learning networks, neural networks may further employ pre-processing of fuzzy input, propagation of results 5 through the network, or interpretation of final results.

Propagation of results may employ max-min networks, learning algorithms for interval data, or analogue models. Unsupervised learning methods may employ fuzzy associative memories or fuzzy clustering. Fuzzy methods for use in clustering include 10 relation criterion functions, object criterion functions, fuzzy isodata, convex decomposition, numerical transitive, generalized nearest neighbor rules, or HCM/FCM clustering algorithm.

Uncertain information in knowledge-based system may employ fuzzy techniques 15 when dealing with uncertainty in relation to input data, knowledge base, inference engine (e.g., binary logic engines or fuzzy logic engines), evidential reasoning (e.g., possibility theory, probabilistic approaches, or Dempster-Shafer Belief Theory), compatibility indices, or approximate reasoning.

20 Alternatively systems-biology platform 104 may employ probabilistic systems or statistical analysis for analysis of medical data. Probabilistic systems may include Bayesian approaches, parameter estimation, discriminant analysis, statistical pattern classification, unsupervised learning, or regression analysis.

Bayesian approaches may include Bayes' Rule, Bayes' Decision Theory, risk analysis, supervised Bayesian learning, or decision trees. Parameter estimation may include maximum likelihood estimation or Bayesian estimation. Unsupervised learning 5 may include Parzen window approach, nearest-neighbor algorithm, mixture densities and maximum likelihood estimates, unsupervised or Bayesian learning.

For example systems-biology platform 104 receives raw data from sensor unit 111 and employs neural networks, artificial intelligence, fuzzy systems, or probabilistic 10 systems to aid in medical decision making for therapy recommendations or diagnosis.

Optionally additional information or test data helpful for diagnosis or treatment may be gathered from electronic files or user input from an outside source via and stored in data storage 105. Additional information or test data may include: patient age, height, 15 weight, symptoms, allergies, diet, previous or present medications, medical or family history of disease, sickness or infection, results of previous blood, urine or other bodily fluid analysis, or other nongenetic (e.g., environmental) or immunological factors relating to the patient.

20 Optionally systems-biology platform 104 sends therapy recommendations or diagnosis report to an outside source via wireless communication 106 and store recommendations or report in data storage 105.

In clinical, managed-care, hospital, diagnostic, therapeutic, or biomedical application or embodiment, systems-biology platform 104, using one or more firmware, source or object code software, configurable logic chip or device, digital signal processor, systolic processing array, or other finite state machine, actually or effectively may

5 compare set of bioinformatic values associated with sensor signal or simulation data, preferably associated with same or different temporal states, to determine or otherwise recognize one or more genomic mutation associated with or corresponding to target patient, animal, plant, or other biological host.

10 Furthermore systems-biology platform 104 may operate autonomously, in cooperation with other computer system nodes, clients, or processing elements, to collect, process and display various host or patient sensor or simulation data, preferably in combination.

15 For example patient information and other personal or medical record data may be received via questionnaire or otherwise retrieved, such as host identification, drug treatment, prescription, and dosage, single or multiple concomitant food or drug allergy, interaction or side effect, pregnancy, lactation, as well as bioinformatic, genetic, proteomic, metabolomic, and other monitored, simulated or sensed mutation-related data

20 as described herein.

Systems-biology platform 104 may be used in time-critical emergency, urgent, or trauma situation to improve patient health-care diagnosis and treatment, for example, by

early-detection, expediting and assisting physician, paramedical, nursing, or other professional analysis and treatment.

Sensed signal or simulated data as electronically may be labeled for indicating 5 genomic mutation, significantly improves quality and accuracy of medication delivery and administration to identified subgroups of patients having certain adverse response to medication, food, or other treatment.

Additionally such data or signal may include pharmaco-genomic or pharmaco-10 kinetic clinical or indications based on genetic, proteomic, metabolomic (i.e., analysis of small organic cell molecules and metabolic response thereof), or other bioinformatic variant or mutation, or other genetic-based condition or profile (e.g., sex, race/ethnicity, etc.) such as drugs to be avoided, or considered as alternative. Thus optimally host susceptibility or predisposition to toxicity or other adverse host reaction or side effects to 15 certain identified food, drugs, or other medical treatment may be minimized, mitigated, or eliminated using automated rule-based advise or expert system.

For example, systems-biology platform 104 may alert medical professionals when 20 host patient is determined via sense or simulation approach to detect genomic mutation that patient ability to produce thiopurine S-methyltransferase (TPMT) enzyme activity is compromised. TPMT genetic test (commercially available from DNA Sciences (Raleigh, NC) enables identification of patient at risk for 6-MP/azathioprine/thioguanine toxicity,

and improves confidence through tailored dosing regimens, while minimizing concern over drug-induced complication.

Alternatively, genomic mutation to G protein-coupled receptors (GPCR) 5 molecular target and variant alleles may be detected to electronically label and thereby effectively modify host drug therapy. Another genomic mutation that may be detected and labeled is enzyme debrisoquine hydroxylase (CYP2D6), isozyme of microsomal cytochrome P450 monooxygenase system; encoding gene is located in CYP2D gene cluster in contiguous 45-kb region of chromosome 22. Here, at least nine polymorphisms 10 of CYP2D6 affect metabolism of more than 30 different pharmaceuticals, including β -adrenergic receptor antagonists, neuroleptics, and tricyclic antidepressants.

Systems-biology platform 104 may couple electronically or digitally to hospital, physician, nursing, or other medical staff communication system to enable network- 15 accessible prescription renewal, appointment scheduling, lab-result entry or retrieval, referrals to specialists and disease management, as well as generally computerized physician or pharmacy-ordering scheme, patient communications, access to medical simulation, test or sensor results, insurance claim status, and bar-coding of pharmaceuticals, and automated medication checks for possible errors.

20

System-biology platform 104 may employ simple identical or substantial equivalent value check between recently-measured value and previously-stored value for same host, for example, after host exposure to radiation or other carcinogenic sources.

Such algorithm may be executed to adapt iteratively or dynamically in real-time or in multiple or parallel processors based on currently or recently-measured, monitored, or sensed host bioinformatic values, for example using fuzzy system, Bayesian or neural network, to improve compute or processing performance by comparing initially values 5 that previously are known or recorded to be related or likely to be related or otherwise weighted to sensor signal or simulation data.

Additionally electronic access to sensor signal or simulation data may be restricted, secured, encrypted, or excluded unless the host thereof explicitly or voluntarily 10 provides prior informed consent to access such information.

Hence, comparison serves to detect presence or absence of target sensor signal, simulation data or other genomic or bioinformatic value (e.g., oncogene, tumor suppressor gene, allele, enzyme, repeat sequence, micro-deletion, or other mutant gene 15 product, protein, or metabolome) that causes, or increases or decreases risk of one or more host disease, disorder, syndrome, allergy, or other biological condition.

Such simulation data or sensor information may be stored in data storage 105 or in other digital storage accessible or otherwise retrievable through network 103. Such 20 stored information may be formatted according to one or more conventional, industry-standard, or otherwise publicly or commercially-available software, processing, storage, and communications protocol, as well as databases for metabolic, signaling, regulatory and pathway data.

Additionally other genomic relational or object-oriented knowledge base or data sources may be network-accessed, such as GenBank, Unigene, LocusLink, Homologene, Ensemble, GoldenPath, or NCICB Cancer Genome Anatomy Project (CGAP). Such 5 information may be accessed using ontology-based interfaces that are defined to be logically related, for example, using annotation format such as Distributed Annotation System (DAS).

Optionally systems-biology platform 104 data or instructions may be specified 10 and otherwise annotated, such as hypothesis definition, experiment design, sample preparation and distribution, experiment run, data acquisition, result analysis, data mining, design refinement, modeling, knowledge discovery, or project report. Additionally such functions may be applied to simulation data or sensor signal processed by software or hardware analysis tools, e.g., for pharmacogenomics, gene expression, 15 high-throughput sequencing, or proteomics (functional or structural) use-case domains.

Preferably such stored information complies, at least in part, with data exchange and management framework and specifications provided by Interoperable Informatics Infrastructure Consortium (I3C), which technical and use-case model documents, and 20 recommended implementations, as described on-line at <http://www.i3c.org/> are hereby incorporated by reference as appropriate herein.

For example, one or more I3C-compliant or recommended data format may be employed during operation of electronic label processor, as described herein. Accordingly simulation data or sensor signal may be accessed, and displayed or otherwise imaged using electronic display I/O hardware or software, for gel 5 chromatography images, original data from biological arrays, arrays of time-series data from mass spectrometry, illustrative functional depiction of proteins, simple microscope images, patient records with medical images, derived data from multiple or time-series images, electrocardiograms, or original drawings and annotations to medical images made by examining professionals. On-screen search capability enables medical 10 professional quickly to locate and interpret particular host simulation data or sensor signal, such as gene sequence, protein, enzyme, allele, or other related detail.

Network 103 access to various databases or other digital repository may couple in n-tiered architecture multiple client interfaces, serve components, back-end objects and 15 data sources. For example, Linux-based, Netscape, or Microsoft Internet Explorer browsers or applications, e.g., based on Java, non-Java, Perl, C, C++, or other programming or development software, run on client nodes 60 may receive information, such as in various markup-language, e.g., HTML, XML, etc., from back-end objects over conventional network messaging or transport protocol, e.g., hyper text transfer protocol 20 (HTTP), TCP Internet Protocol, simple object access protocol (SOAP), file transfer protocol (FTP), IIOP, etc. Additionally Universal Description Discovery Integration (UDDI) registry and Resource Description Framework (RDF) agent advertising formats may be used.

Further genomic, proteomic, or metabolomic sequence analysis software tool, for example, (e.g., BLAST, TimeLogic) may be used by controller 112 to discover or characterize host genomic, proteomic, or metabolomic sequence, acquired and qualified 5 from one or more sources, such as sensor unit 111 or data storage 105. Thus, internal and external sequence and protein libraries may be updated and maintained, certain redundant, unqualified or external data being filtered for internal sequence processing. One or more target, putative or otherwise mutant gene or bioinformatic value is then determined and cataloged effectively by systems-biology platform 104.

10

Hypothetical function of such determined gene or value may be generated manually, automatically, or homologously by finding similarity to known or other prior values. Genetic, proteomic, or metabolomic analysis protocols and similarity analysis may be defined and selected, thereby enabling or constructing functional hypotheses to 15 be generated, prioritized, or reviewed using sensor measurements or other host evidence.

Proteolysis sample preparation may be performed (e.g., HPLC, gel electrophoresis), then mass spectroscopy or tandem MS analysis and compression, quantitization, and fragment size genome analysis for candidate prediction, proteome or 20 metabolome comparison, and other quantitative analysis using modeling tools and databases.

Systems-biology platform 104 may receive data from sensor unit 111, and neural networks, artificial intelligence, fuzzy systems, or probabilistic systems consider presence of conditions in diagnosis of genetic disorders: point mutations, mutations within non-coding sequences, deletions and insertions, trimucleotide repeat mutations, autosomal 5 mutations, gain of function mutations, loss of function mutations, mutations in mitochondrial genes, enzyme defects, defects in receptors and transports systems, defects in receptors and transport systems, alterations in structure, function or quantity of non-enzyme proteins, defects in receptor proteins, defects in protooncogenes or tumor-suppressor genes, aneuploidy, unbalanced autosome, sex chromosome abnormality, 10 fragile X syndrome, ring chromosome, chromosome inversion, isochromosome formation, translocation, or abnormal gene products.

Optionally allele-specific oligonucleotide hybridization may be employed in multifunctional array 200 in biosensor platform 102 to assist in direct gene diagnosis of 15 mutations. Systems-biology platform 104 may diagnose genetic disease or mutation, such as Mendelian disorders, autosomal dominant disorders, autosomal recessive disorders, X-linked disorders, Marfan syndrome, Ehlers-Danlos syndrome, familial hypercholesterolemia, lysosomal storage diseases, Tay-Sachs Disease, Gangliosidosis, Niemann-Pick disease, Gaucher Disease, glycogen storage diseases, 20 Mucopolysaccharidoses, Alkaptonuria, Neurofibromatosis, trisomy 21, chromosome 22q11 deletion syndrome, Klinefelter syndrome, XYY syndrome, Turner Syndrome, Multi-X females, hermaphroditism, pseudohermaphroditism, triplet repeat mutations,

chromosome-breakage syndrome, Prader-Willi syndrome, Angelman syndrome, or gonadal mosaicism.

Alternatively, systems-biology platform 104 may diagnose infectious disease or infection, such as *Haemophilus influenzae* infection, tuberculosis, histoplasmosis, coccidioidomycosis, *shigella* bacillary dysentery, *Campylobacter enteritis*, *Yersinia enteritis*, *Salmonellosis*, typhoid fever, cholera, amebiasis, giardiasis, herpes, chlamydia, gonorrhea, syphilis, trichomoniasis, staphylococcal infection, streptococcal infection, clostridial infection, measles, mumps, mononucleosis, polio, chickenpox, shingles, 10 whooping cough, diphteria, infections associated with Neutropenia and Helper-T cell depletion, cytomegalic inclusion disease, *pseudomonas* infection, legionnaires disease, listeriosis, candidiasis, cryptococcosis, aspergillosis, mucormycosis, pneumocystis pneumonia, cryptosporidium and cyclospora infection, toxoplasmosis, dengue fever, Rickettsial Infection, trachoma, leprosy, plague, relapsing fever, lyme disease, malaria, 15 babesiosis, leishmaniasis, African Trypanosomiasis, Chagas disease, Trichinellosis, hookworm, cysticercosis, Hydatid disease, schistosomiasis, lymphatic filariasis, or onchocerciasis.

In diagnosing infectious disease or infection, systems-biology platform 104 receives data from sensor unit 111 or neural networks, artificial intelligence, fuzzy systems, or probabilistic systems that consider presence of infectious agent, such as a prion, virus, bacteriophage, plasmid, transposon, chlamydiae, rickettsiae, mycoplasma, fungi, protozoa, helminths, or ectoparasite. In host system, systems-biology platform 104 may also consider the presence of bacterial endotoxin, bacterial exotoxins, proliferation

and morphologic lesions of epithelial cells, tissue necrosis, granulomas, cysts, increased levels of leukocytes, mononuclear cells or neutrophils, mononuclear interstitial infiltrates, reduced levels of immune cells (e.g. cytokines, lymphocytes, macrophages, dendritic cells or natural killer cells), bacterial leukotoxins, hemagglutinin, spores, or other antigen 5 or protein from bacteria, virus, fungi, protozoa, or parasite.

Alternatively systems-biology platform 104 may diagnose disease of immunity, such as hypersensitivity disorders (immune complex mediated, complement-dependent reactions, cell mediated, or anaphylactic type, transplant rejection), autoimmune disease, 10 systemic sclerosis, inflammatory myopathies, mixed connective tissue disease, polyarteritis nodosa or other vasculitides, X-linked agammaglobulinemia of Bruton, common variable immunodeficiency, isolated IgA deficiency, Hyper IgM syndrome, DiGeorge syndrome, severe combined immunodeficiency disease, immunodeficiency with thrombocytopenia and eczema, acquired immunodeficiency syndrome (AIDS), or 15 amyloidosis.

In diagnosing immunity diseases, systems-biology platform 104 considers following sensed, detected, or measured conditions from sensor unit 111: levels of immune cells (e.g., mast cells, cytokines, lymphocytes, macrophages, dendritic cells or 20 natural killer cells), MHC (major histocompatibility complex) molecules or antigens, HLA (human leukocyte antigen) complex, antigens, or types, or levels of primary mediators (e.g., biogenic amines, chemotactic mediators, enzymes, or proteoglycans), secondary mediators (e.g., leukotrienes, prostaglandins, platelet-activating factors, or

cytokines), histamines, platelet-activating factor (PAF), neutral proteases, chemotactic factors, or antigen-presenting cells (APC).

In diagnosing autoimmunity diseases, systems-biology platform 104 receives data
5 from sensor unit 111 or neural networks, artificial intelligence, fuzzy systems, or probabilistic systems considers presence of auto-antibodies disease and considers whether auto-antibodies are directed against single organ or cell type or whether it is systemic. Autoimmune diseases include single organ or cell type related diseases (e.g., hashimoto thyroiditis, autoimmune hemolytic anemia, autoimmune atrophic gastritis of
10 pernicious anemia, autoimmune encephalomyelitis, autoimmune orchitis, goodpasture syndrome, autoimmune thromcytopenia, insulin-dependent diabetes mellitus, myasthenia gravis, Graves disease), or systemic autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis, Sjögren syndrome, or Reiter syndrome).

15 Systems-biology platform 104 may identify whether disease condition may be single organ or cell type autoimmune diseases or primary biliary cirrhosis, chronic active hepatitis, ulcerative colitis, or membranous glomerulonephritis. The platform is also identifies whether disease condition may be systemic autoimmune disease or inflammatory myopathies, systemic sclerosis (scleroderma) or polyarteritis nodosa.

20

Furthermore systems-biology platform 104 may determine presence of pathologic autoimmunity by considering at least three requirements, such as presence of autoimmune reaction, clinical or experimental evidence that such reaction is not

secondary to tissue damage but of primary pathogenetic significance, or absence of another well-defined cause of disease.

Alternatively systems-biology platform may be used in diagnosis of neoplasia. In 5 diagnosing neoplasia, systems-biology platform 104 receives sensed, detected, or measured data from sensor unit 111 and neural networks, artificial intelligence, fuzzy systems, or probabilistic systems considers the following factors: DNA damage, failure of DNA repair, mutations in the genome of somatic cells, activation of growth-promoting oncogenes, alterations in the genes that regulate apoptosis, inactivation of cancer 10 suppressor genes, expression of altered gene products and loss of regulatory gene products, oncoproteins, growth factors, growth factor receptors, proteins involved in signal transduction, nuclear regulatory proteins, cell cycle regulators, tumor antigens, or the levels of immune cells (e.g., mast cells, cytokines, lymphocytes, macrophages, dendritic cells or natural killer cells).

15

Systems-biology platform 104 may consider epidemiological factors in determining diagnosis for neoplasia. Epidemiological factors may include cancer incidence, geographic or environmental factors (DNA damaging agents - e.g. chemicals, radiation or viruses), or heredity (e.g., inherited cancer syndromes, familial cancers, 20 autosomal recessive syndromes of defective DNA repair). Systems-biology platform 104 may consider tumor markers such as hormones (e.g. human chorionic gonadotropin, calcitonin, catecholamine and metabolites, or ectopic hormones), oncofetal antigens (α -fetoprotein or carcinoembryonic antigen), isoenzymes (e.g., prostatic acid phosphatase, or

neuron-specific enolase), immunoglobulins, prostate-specific antigens or mucins or other glycoproteins (e.g. CA-125, CA-19-9, or CA-15-3).

After systems-biology platform 104 makes diagnosis, platform may recommend
5 treatments in combination or individually. Such recommendation may include diet changes, surgery, radiation therapy, chemotherapy, medications, antiangiogenesis therapy, or other cancer treatment. Systems-biology platform 104 may instruct therapeutic unit 113 to manufacture or dispense pharmaceuticals, biopharmaceuticals, or other therapeutic tools for the treatment of neoplasia.

10

Systems-biology platform 104 may employ sensor device and simulation method for analyzing dynamic hormone-secretion phenomena in dynamic biological systems, for example using sensor, artificial neural network, and dosing device; e.g., Sicel Technologies wireless or telemetric sensor platform for measuring parameters of relevance in vivo, such as radiation dose, tissue microenvironment or gene expression to increase treatment success. Implantable sensors may be provided 2mm diameter, 15mm length, for injection at margin of tumors using minimally invasive procedure.

20 Biosensor 100 may be applied to food technology, e.g., pasteurization or development or production of artisan foods. DNA sensor 201 may monitor, detect, or measure amount of bacteria or microflora used to ripen and develop flavors in foods, such as artisan cheese. Similarly peptide or protein sensor 203, lipid or fatty acid sensor

208, or small molecule sensor 217 may monitor bacterial or microflora production of fats, proteins, esters, or other biologically-active molecules.

5 Biosensor 100 may be applied to food manufacturing industry, e.g., quality control, food safety, or countering food borne illness caused by bioterrorism. Biosensor 100 may detect types of food contaminants, including bacteria or chemicals that cause human sickness, or counter bioterrorism acts threatening consumer food supply.

10 Biosensor 100 may be used by food manufacturer, crop cultivator, lab researcher, consumer, packer, distributor, receiver, food vendor, or food inspector to ensure quality control and food safety. Biosensor platform 102 may detect, measure, or determine presence or absence of parasitic organism, virus, bacteria, fungi, protozoa, or unicellular or multi-cellular organism present during food manufacturing process or growth of food crops, or prior to consumption.

15

Chemical sensor 216 may be used to sense, detect, or measure foreign chemicals, such as toxins, vitamins, minerals or other organic and inorganic chemicals. Systems-biology platform 104 may analyze raw data from biosensor platform 102 to identify potentially-hazardous organism or chemical or flag unknown organism or chemical.

20

When systems-biology platform 104 identifies or quantifies potentially hazardous organism or chemical or unknown organism or chemical, data is stored in storage 105. Systems-biology platform 104 may generate report document or electronic multi-media

warning or signal, which discloses detected organism or chemical and determine whether manufacturing, crop growth, or consumption is safe to continue.

Systems-biology platform 104 may send automated warning or signal, sent via
5 wireless communication 106, to information recipient interested in data gathered by the platform, such as remote database, researcher, lab, government agency, or health or safety maintenance organization.

Chemical sensor 216 may determine purity or verify amount of vitamin, mineral,
10 herb, or botanical claimed by a food product, meal supplement, vitamin supplement, or other nutritional substance. Systems-biology platform 104 may compare amount of vitamin, mineral, herb or botanical determined by chemical sensor 216 to pre-set amount or range stored in storage 105, e.g. amount or range determined by government agency or health or safety maintenance organization.

15

Systems-biology platform 104 generates report whether detected amount or range complies with pre-set amount or range, and determines whether manufacturing or consumption is safe to continue. Detected amount can be reported and sent via wireless communication 106 to outside source or information recipient interested in data gathered by chemical sensor 216, such as packer, distributor, receiver, remote database, researcher, lab, government agency, or health or safety maintenance organization. During
20 manufacturing, determined amount of vitamin, mineral, herb, or botanical present in each

lot or batch of produced product is recorded or accessible through network 103 for analysis.

5 Optionally if amount of vitamin, mineral, herb, or botanical falls outside pre-set amount or range, systems-biology platform 104 generates automated warning to outside source or information recipient. Biosensor 100 monitors manufacturing of food product, meal supplement, vitamin supplement, or other nutritional substance by ensuring that manufactured substance complies with required amount or range of nutritional substance. Chemical sensor 216 may be used to demonstrate whether particular vitamin, mineral, 10 herb, botanic, or other natural or organic food has properly absorbed in biological system of organism.

15 Biosensor 100 may synchronize different input stimuli, particularly with integrated purpose of evaluating food and drug interactions positively or negatively within host. Systems-biology platform 104 can analyze genetic composition of host, determined through DNA sensor 201, to assist in predicting particular drug-food interactions. To assist in predicting drug and food interactions, host genetic composition may be supplemented with additional information or test data including nongenetic (e.g. 20 environmental, epidemiological) or immunological factors relating to host.

20

 Biosensor 100 may be implanted within a host and pharmacogenetics 304 or pharmacokinetics 308 in systems-biology platform 104 may be employed to monitor or determine activity or effectiveness of medication used individually or in combination. Meanwhile, biosensor 100 placed remotely or separately from implanted biosensor is

used to analyze nutritional substance (e.g., food product, meal supplement, vitamin, or mineral) that may be consumed by same host.

5 Data from remote biosensor 100 is coupled, received, or combined to data from implanted biosensor or analyzed collectively by systems-biology platform 104 to predict or model combined allergic reactions, side effects, or adverse reactions that result from consumption of nutritional substance in conjunction temporally with related medication.

10 Systems-biology platform 104 may generate automated recommendation or report diagnostically or therapeutically about optimum level of nutritional substance or identify alternative substance for consumption. Data from remote and implantable biosensor data, and recommendation or determination processed by systems-biology platform 104 may be stored in data storage 105. An outside source or information recipient may access data and results in data storage 105 through wireless communication 106 for analysis via 15 network 103.

20 When systems-biology platform 104 identifies nutritional substance that may cause an adverse or positive reaction, automated warning or message may be transmitted wirelessly to information receipt interested in the gathered data. The ability of systems-biology platform to analyze or model nutritional substance and host condition in combination using host sensor data and consumable sensor data optimizes treatment of real-time physiological condition.

Biosensor 100 may be applied to biopharming purpose, e.g., field tests or inspections of genetically engineered plants, and use of genetically engineered plants or transgenic crops to produce therapeutic proteins and industrial enzymes with safeguards for ensuring that food crops are not co-mingled with food crops intended for 5 pharmaceutical or industrial use.

To prevent out-crossing or commingling of genetic material, DNA sensor 201, RNA sensor 202, or peptide and proteins sensor 203 in biosensor platform 102 may detect, sense or measure presence or absence of foreign genetic material or protein in 10 food crop not intended for pharmaceutical or industrial use. Systems-biology platform 104 may analyze raw data from biosensor platform 102 to identify out-crossing or commingling of genetic material.

When systems-biology platform 104 identifies foreign genetic material, data is 15 stored in storage 105. Systems-biology platform 104 may generate report about detected foreign genetic material or determine whether crop growth is safe to continue. Systems-biology platform 104 may send automated warning or signal, via wireless communication 106, to information recipient interested in data gathered by platform, such as remote 20 database, researcher, lab, government agency, or health or safety maintenance organization.

Biosensor 100 may monitor growth of food crops, e.g., sensors (e.g. peptide or protein sensor 203, vector or virus vector sensor 207, pH sensor 212, metabolites sensor

219, etc.) in biosensor platform sensor 201 may sense, detect or measure abnormalities in crop growth or reproduction. Biosensor 100 may monitor, detect or measure pesticides, insecticides or foreign chemicals effect on growth or reproduction.

5 Biosensor 100 may be applied to bio-manufacturing industry, e.g., drug-producing plants and transgenic animals, such as cows genetically transformed to excrete different kinds of therapeutic proteins in breast milk. Peptide or protein sensor 203 in biosensor platform 102 or antibody sensor 204 may detect or measure presence or absence of genetically engineered therapeutic protein or antibody in breast milk or other
10 biological fluid.

15 Biosensor 100 may be applied in xenotransplantation, for example by screening animal organs for transplantation into humans. Sensor unit 111 senses, measures, or processes biological molecule, such as cell, tissue, or intracellular or extracellular material from animal cell, tissue or organ, or raw data is analyzed by system biology platform 104. System biology platform 104 analyzes or determines whether animal cell, tissue, or organ is compatible for use with human for transplantation or other therapeutic process.

20 Biosensor 100 may be applied to avian transgenics, particularly to proteins produced through poultry-based production systems. For example, biosensor platform 10 may detect whether successful transformation is occurring via avian embryonic germ

cell, retroviral-mediated transformation, sperm-mediated transgenesis, avian embryonic stem cell, direct egg transfection, or other transformation process.

Biosensor 100 may be applied to drug-producing plants, e.g., tobacco, corn, or 5 other non-food plants, for biomanufacturing. Peptide or protein sensor 203 may detect, sense or measure presence, absence, manufacture or biological activity of recombinant proteins manufactured in plants. DNA sensor 201, RNA sensor 202, vector or virus vector sensor 207, chromosome sensor 221, or cell sensor 222 may monitor or detect 10 whether genetic material, vector, chromosome, or cell successfully integrates or genetically transforms plant or animal.

Figure 3c systems-biology platform 104, therapeutic unit 113, and sensor unit 111. Systems-biology platform 104 provides verification of data 321, to assure that data is proper or feasible from biosensor platform 102 within sensor unit 111. Verification of 15 data 321 identifies sequence or structures of target system. Data may be analyzed statistically by systems-biology platform 104, using statistical computation, e.g., scatter plot matrices, Venn diagrams, comparative histograms, volcano plots, or gene ontology charts. Computed statistics are interpreted biologically, filtering or reducing dataset to manageable size by eliminating results that show insignificant or uninteresting biological 20 data.

Verification of data 321 includes checking regulatory relationship of genes or interaction of proteins that provide signal transduction or metabolism pathways, as well

as physical structure of organisms, organelle, chromatin, cell-cell interactions, or other components.

To integrate sensor data, software and management systems are used. Systems-biology platform 104 may utilize management software, e.g., Analysis Information 5 Management System (AIMS), using tools to analyze or manage range of complexity of data obtained from microarrays or assays, tracking computational processes. Data-mining tools, e.g., high-dimensional data analysis tools, may process data where data have multiple dimensions.

10 Data may be formatted using standardization programs, e.g., Gene Expression Markup Language (GEML), Microarray Markup Language (MAML), Microarray and Gene Expression Data (MAGE), MicroArray and Gene Expression Markup Language (MAGE-ML), solutions by by Microarray Gene Expression Database group (MGED) or Minimum Information About a Microarray Experiment (MIAME), or other programs.

15 After data is verified, modeling/simulation 322 uses combined simulation data or sensor signal to model biological structures or relative interactions. Modeling or simulation 322 simulates biological interactions to identify behavior of system, for example, sensitivity of behaviors against external perturbations and how quickly system returns to normal state after stimuli.

20 Another example includes simulating how individual malfunctioning mis-folded protein interacts with other proteins or cellular components, with simulations on how protein responds to particular therapies; yet another example is modeling phospho-proteomics and systems biological role for oncology target discovery or validation.

Modeling or simulation 322 predicts methods of controlling state of biological system, e.g., pharmaceutical or gene therapy transformation of malfunctioning cells into healthy cells. For example through structural analysis, regulation of c-Abl and ST1-571 specificity may be achieved.

5 Modeling or simulation 322 prediction is translated into instructions for therapeutic unit 113 to implement appropriate therapy to fix biological system. These instructions are conveyed to therapeutic unit 113, where instructed therapy may be performed.

10 Sensing unit 111 monitors progress, efficiency, or ancillary effects of induced therapy on biological system. Data from sensing unit 111 may be verified by verification of data 321, which provides cyclical self-regulating process.

15 Figure 4a shows flow of instructions from systems-biology platform 104 to network 103 to components comprising therapeutic unit 113. Components of therapeutic unit 113 include therapeutic manufacture 108, therapeutic reservoirs 109, and reconfigurable sensor manufacture 110. These components may be reconfigurable or software-programmable according to systems-biology platform 104, or from external source through wireless communication unit 106.

20 Figure 4b shows therapeutic manufacture 108 of: pharmaceuticals 401, biopharmaceuticals 402, tissue, reconfigurable biocatalytic chips 403, tissue scaffolds 404, M/N machines 405, or other therapeutic material or tools. These components may be reconfigurable and software-programmable according to systems-biology platform 104, or from external source through wireless communication unit 106.

Pharmaceuticals 401 may be known and matched with organism, or computationally derived or optimized from systems-biology platform 104. Pharmaceuticals 401 is defined herein as including chemical substance that provides benefit to system.

5 Biopharmaceuticals can be naturally-occurring biological molecule or structural derivative of biological molecule. For example, biopharmaceuticals can be isolated DNA molecules, recombinant DNA molecules, DNA fragments, oligonucleotides, antisense oligonucleotides, RNA molecules or constructs, self-modifying RNA molecules, catalytic RNAs, ribozymes, modified ribozymes, synthetic peptides, peptide linkers, proteins, 10 fusion proteins, antibodies, modified antibodies, antigens, cell surface receptors, monoclonal antibodies specific for epitopes, polyclonal antibodies, tissue factors, modified tissue factors, mutant tissue factors, ligands, vectors, virus strains for gene transfer, recombinant plant viral nucleic acids, bacterial strains, oil-body proteins as carries of high-value peptides in plants, host cells, transformed cells, or microorganisms 15 newly isolated in pure form from natural source.

Therapeutic unit 108 may prepare biopharmaceutical product such as 2 μ g of sub50-nm tenascin nanocapsules containing antisense of protein kinase CK2 α subunit or similarly GFP and RFP-labeled bacteria which produce toxins or other therapeutic proteins to be used to target tumors. Further therapeutic unit 108 can perform functions 20 like so-called Intelligent Pill (e.g., University of Calgary) in which information relayed to chip that controls micropumps that squeeze-out therapeutic material.

Therapeutic manufacture unit 108 may prepare therapy comprising pharmaceutical 401 or biopharmaceutical aspect 402. For example antiangiogenesis therapy using yttrium-90 nanoparticles with conjugated anti-Flk-1 monoclonal antibody administered by i.v. injection is anti-angiogenic agent for treatment of solid tumors.

5 Therapeutic manufacture unit 108 may produce small interfering RNA (siRNA) used to inhibit P-gp encoded by *MDR1* gene; production enhances accumulation of sensitivity of multidrug-resistant cancer cells to drugs transported by P-glycoprotein.

Reconfigurable biocatalytic chips 403 are software programmable from instructions by systems-biology platform 104, or from external source through wireless communication unit 106. Depending on instructions, reconfigurable biocatalytic chips 403 can be activated, deactivated, manufactured, or disassembled. Reconfigurable biocatalytic chips 403 undergo molecular bioprocessing, fabricating or manipulating single and multienzyme systems on biochip to induce artificially biocatalysis in system.

Tissue scaffolds 404 may be reconfigurable, and controlled by systems-biology platform 104 instructions (or from external source through wireless communication unit 106). Scaffold 404 may be substrate to grow cells or tissues, which may be activated or deactivated according to signaled instructions. Permanent or biodegradable tissue scaffolds can be used. Further scaffold 404 may be personalized by systems-biology platform, e.g., John Hopkins University stem cell-based polymer scaffolds for tissue engineering using composite hydrogel. After modeling tissue development on biomaterial scaffolds based on individualized systems-biology profile, reconfigurable scaffold 404 can be programmed with biological signals based on individual need.

M/N tools 405 may perform therapeutic treatments, e.g., Johnson & Johnson Cordis Corporation, that make drug coated stents that keep arteries from clogging by releasing medication. Examples of M/N tools may be self-assembling, e.g., Angstrom Medica altered calcium and phosphate molecules that self-assemble to create 5 nanostructured synthetic bone.

Another tool example is S. Stupp project at Northwestern University, which provides long complex molecules with hydrophobic tails and hydrophilic heads; these molecules self-assemble to form cylindrical structures that can be applied to making artificial bone. Another example of M/N tools 405 is Son Binh Nguyen use of 10 nanoparticles for small molecule chemotherapy, in which engineered hydrophobic cyclic peptides attaches to targeted molecules and subsequently chemically react with molecule, breaking it into pieces.

Figure 4c shows components of therapeutic reservoirs 109. Release of therapies is dictated or controlled by systems-biology platform 104 instructions, or from external 15 source through wireless communication unit 106; timing mechanisms or rate of release may be reconfigured by software, e.g., MicroCHIPS implantable bioMEMS for drug delivery, in which silicon reservoirs hold medications in solid, liquid, or gel form, or iMEDD “NanoPORE Membranes,” silicon wafers that have channels or pores with dimensions on nanometer scale for drug release.

20 Pre-filled reservoirs 410 contain medication filled-in biosensor 100 before implantation in living system. Contents of pre-filled reservoirs 410 may be pharmaceuticals or biopharmaceuticals in active form for release directly to living

system. Pre-filled reservoirs 410 may hold probes, amino acids, nucleotides, or building blocks for sensor manufacture 110 for making additional biosensors.

Precursors 411 may be biological and chemical precursors to therapeutic pharmaceuticals and biopharmaceuticals. Depending on instructions from systems-biology platform 104, therapeutic precursors may be released, or therapeutic manufacture 108 may process into active pharmaceuticals or biopharmaceuticals.

Therapeutic storage 412 may store excess medication produced by therapeutic manufacture 108. Application of storing medication rather than manufacturing as needed if large doses, i.e., that cannot be made fast enough by therapeutic manufacture 108, are needed at time intervals.

Figure 4d shows basic components or interactions of sensor manufacture 110. Systems-biology platform 104 sends software instructions to sensor manufacture 110 to dictate manufacture, disassembly, activation, or deactivation of software-programmable biosensors. Once reconfigurable biosensors are programmed and produced, such components and sensor data signals are integrated, multiplexed, or processed in combination into biosensor platform 102 for biological sensing.

Biosensor chip 421 acts as array or probe arranger 420 attaches probes onto array. Probe arranger 420 may attach probe for assaying, according to instructions by systems-biology platform 104. Method of attaching by probe arranger 420 can be printing method (e.g., placing probes on array with automated machinery). Probes may be attached through microspotting, in which automated microarray is produced by printing small quantities of pre-made biochemical substances onto solid surfaces.

Printing method may be ink-jet printing, e.g., GeSiM; non-contact method places probes on array, in which probes are sprayed on surface using piezoelectric or other propulsion to transfer biochemical substances from nozzles to solid surfaces, or directly placed. This method allows *in situ* synthesis, advantageously synthesizing 5 oligonucleotides on-the-fly directly on array surface. To change DNA that may be placed on array, systems-biology platform 104 provides probe arranger 420 list of sequences to synthesize.

Another example of probe arranger 420 is photolithography, e.g., Affymetrix GeneChips. Photolithography allows oligonucleotides to be built base-by-base (e.g., 10 proteins build amino acid-by-amino acid) on array surface by repeated cycles of photodeprotection and nucleotide or amino acid addition. Like ink-jet printing, this process allows building of M/N arrays without preexisting probes and can generate probes *in situ* on surface of biosensor chip 421.

Customizable microarray platform, e.g., CombiMatrix, including semiconductor-based desktop microarray platform may fabricate custom oligonucleotide biochips. Microarrays with unique content are designed and fabricated on-the-fly using software driven process to generate reagents electrochemically. DNA oligonucleotides are synthesized *in situ* according to probe sequence designed. Probe arranger 420 may use 15 cell-positioning chip, e.g., Aviva chip, to provide living whole-cell arrays.

20 Optionally soft lithography may use stamp to pattern surfaces of array, using patterned elastomer based on program instructions to define microfluidic networks on surface.

Figure 5 shows DNA unit 500, representing organization of sensors in biosensor platform 102, such as RNA sensor 202, peptide or protein sensor 203, etc. DNA unit 500 may include DNA sensor 201, DNA therapeutic manufacture 501, DNA therapeutic reservoirs 502, or DNA reconfigurable biosensor 503 together in same physical structure, 5 which lay in close proximity with each other. DNA therapeutic manufacture 501 is structure-specific category of therapeutic manufacture 108. DNA therapeutic reservoirs 502 and DNA reconfigurable biosensor 503 are structure-specific categories of therapeutic reservoirs 109 and sensor manufacture 110 respectively.

Sequential steps begin with input introduction into DNA unit 500, specifically 10 DNA sensor 201. Raw data is transferred to systems-biology platform 104, a remote source. Systems-biology platform 104 processes information, outputting data and giving instructions to DNA therapeutic manufacture 501, DNA therapeutic reservoirs 502, and DNA reconfigurable biosensor 503. DNA therapeutic manufacture 501, DNA therapeutic reservoirs 502, and DNA reconfigurable biosensor 503 perform instructed 15 tasks, with DNA sensor 201 monitoring respective progress.

DNA sensor 201 monitors or senses organism response to therapies dispensed by DNA therapeutic manufacture 501, DNA therapeutic reservoirs 502, or DNA reconfigurable biosensor 503. Proximity of DNA sensor 201, DNA therapeutic manufacture 501, DNA therapeutic reservoirs 502, or DNA reconfigurable biosensor 503 20 within same unit facilitates monitoring from DNA sensor 201.

Ongoing feedback is transmitted from DNA sensor 201 to DNA therapeutic manufacture 501, DNA therapeutic reservoirs 502, and DNA reconfigurable biosensor

503, while responding continually to DNA sensor 201 raw data, creating cyclic system of monitoring or responding.

Figure 6 flow chart shows automated or computer-assisted diagnosis or therapy recommendations or reports for target host, which is identified initially for possible

5 diagnosis or treatment 601. To determine if host benefits from diagnosis or treatment, host undergoes preliminary screening 602. Preliminary screening may be implemented through software form; host undergoes preliminary modeling 603.

Modeling or simulation is used to model appropriate components or
10 characteristics of device. After preliminary modeling 603, behavior of model is verified for accuracy 604. If behavior of model is not ok, biosensor 100 is modified 605, and preliminary modeling 603 is repeated. If behavior of model is ok 604, biosensor 100 is configured 606. Reconfigurable biosensor is made or programmed according to such model.

15

Reconfigurable biosensor may be verified to comply or adhere to FDA regulations 607. If biosensor does not comply or adhere, it is modified 608 and configuration 606 or verification of adherence to FDA regulations 607 is repeated. If biosensor does comply or adhere to FDA regulations, it is implanted or attached to host.
20 609.

Biosensor is initialized to allow sensor or detection activity *in vivo* 610. Sensing or software is executed 611. Initialization of biosensor and execution of sensing or

software may operate in sequential order or in parallel. Once biosensor and software is initialized, initial *in vivo* sensing begins 612. Sensor data is then used for *in vivo* modeling 613 via systems-biology platform 104. After *in vivo* modeling 613, biosensor 100 generates diagnosis or therapy recommendation 614.

5

Therapy recommendations may result in commands to therapeutic unit 615 for therapeutic manufacturing or dispensing. Ongoing feedback between initial *in vivo* sensing 612, diagnosis or therapy recommendations 614, or commands to therapeutic unit creates an automated sensing, modeling, and treatment cycle.

10

Foregoing descriptions of specific embodiments of the invention have been presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the invention to precise forms disclosed. Modifications and variations are possible in light of above teaching.

15

Embodiments were chosen or described in order to explain principles and application of the invention, thereby enabling others skilled in the art to utilize the invention in various embodiments or modifications according to particular purpose contemplated. Scope of the invention is intended to be defined by claims appended 20 hereto and equivalents.

11-06-03



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Fernandez, Dennis S.

Application No.: 10/646,682

Filed: 8/22/2003

Group Art Unit: Not yet assigned

Title: Integrated Biosensor and Simulation
System for Diagnosis and Therapy

Examiner: Not yet assigned

Attorney Docket No.: FERN-P013

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DRAWING TRANSMITTAL LETTER

Sir:

Enclosed herewith please find:

() sheets of redlined drawing(s) which indicate proposed changes to the drawing(s). Upon approval of these proposed changes, formal drawing(s) will be submitted.

() sheets of corrected formal drawing(s), as required by the Notice of Patent Drawings Objection (PTO-948) which accompanied the Office Action dated

() sheets of corrected formal drawing(s), as required by the Notice of Patent Drawing(s) Objection (PTO-948) and approved in the Notice of Allowability dated

(X) 12 sheets of formal drawing(s). Please substitute these formal drawing(s) for the informal drawing(s) originally filed.

(X) 1 A self-addressed stamped return postcard.

Examiner's approval of the entry of these drawings is respectfully requested. No new matter has been added.

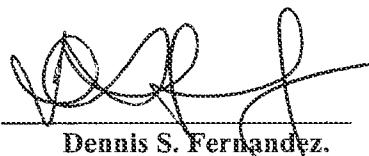
I hereby certify that this correspondence is being deposited with the United States Postal Service as priority and express mail with mailing label number of EV 071301335 US in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Date of Deposit: 11/4/03

Typed Name: Mary Chow

Signature: Mary Chow

Respectfully Submitted,

By 
Dennis S. Fernandez,
Attorney for Applicant(s)
Reg. No. 34,160
CUSTOMER NO: 22877

Date: 11/4/03
Phone: (650)-325-4999

Implantable Network Biosensor 100

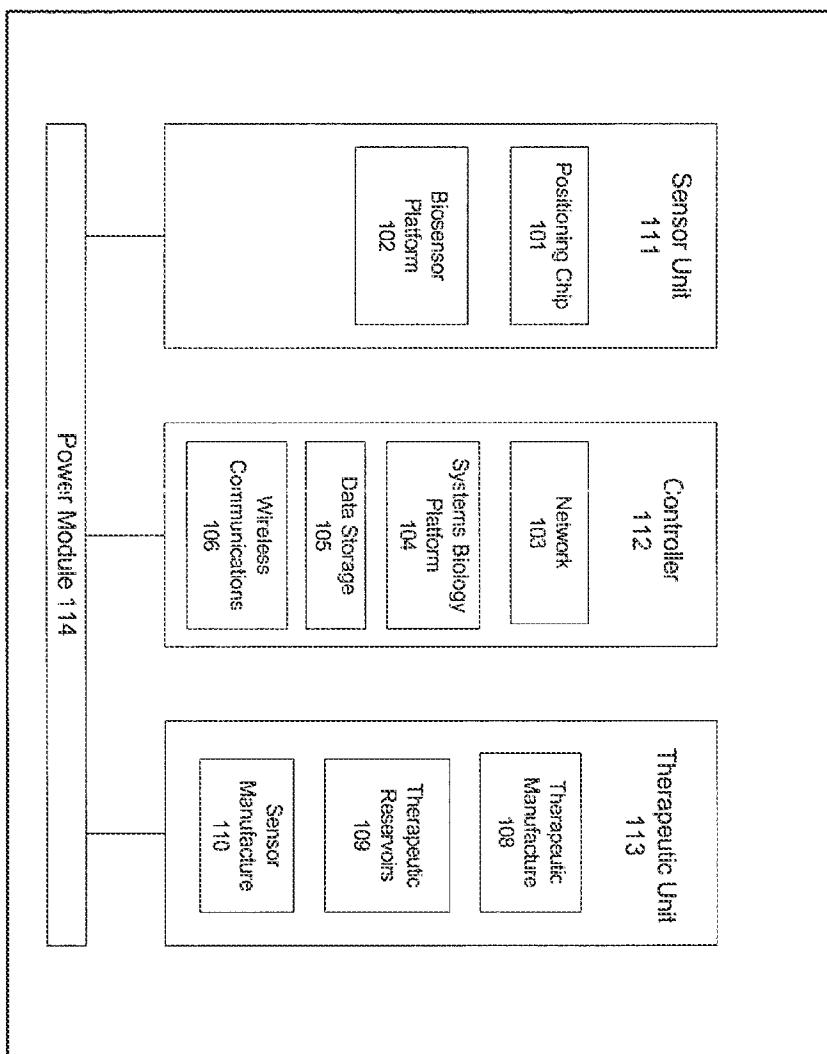


Figure 1a



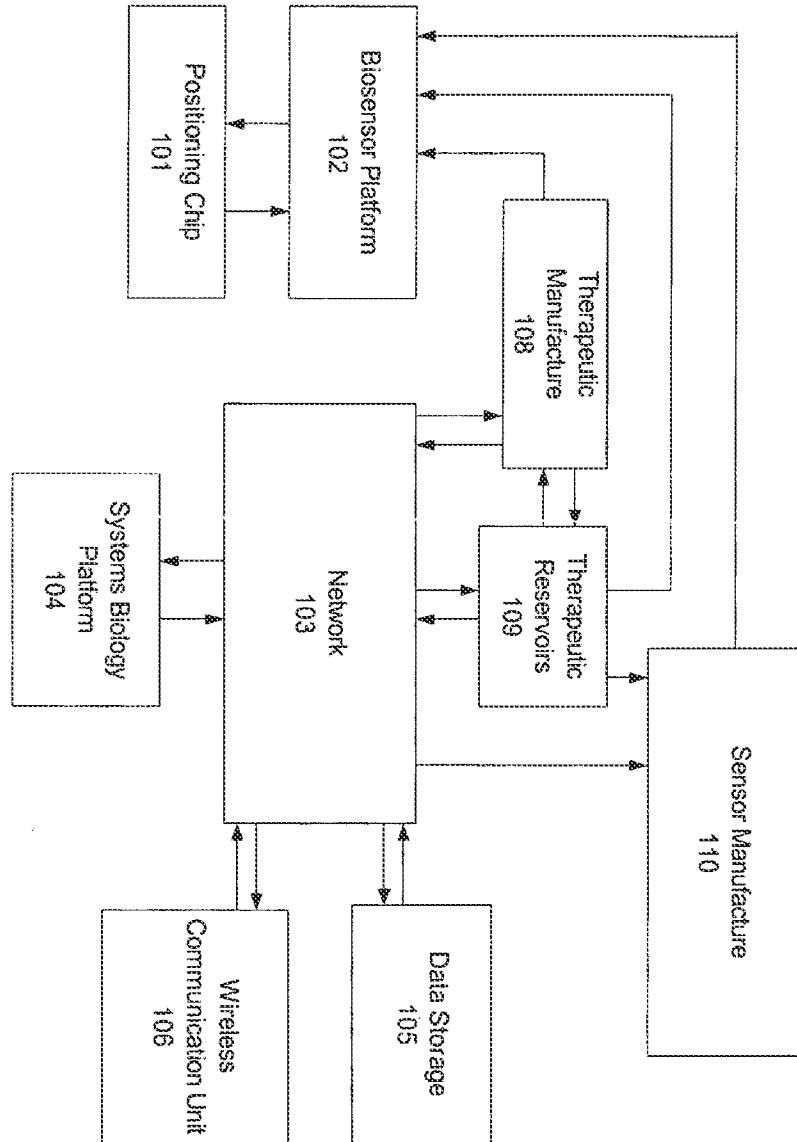


Figure 1b



Biosensor Platform 102

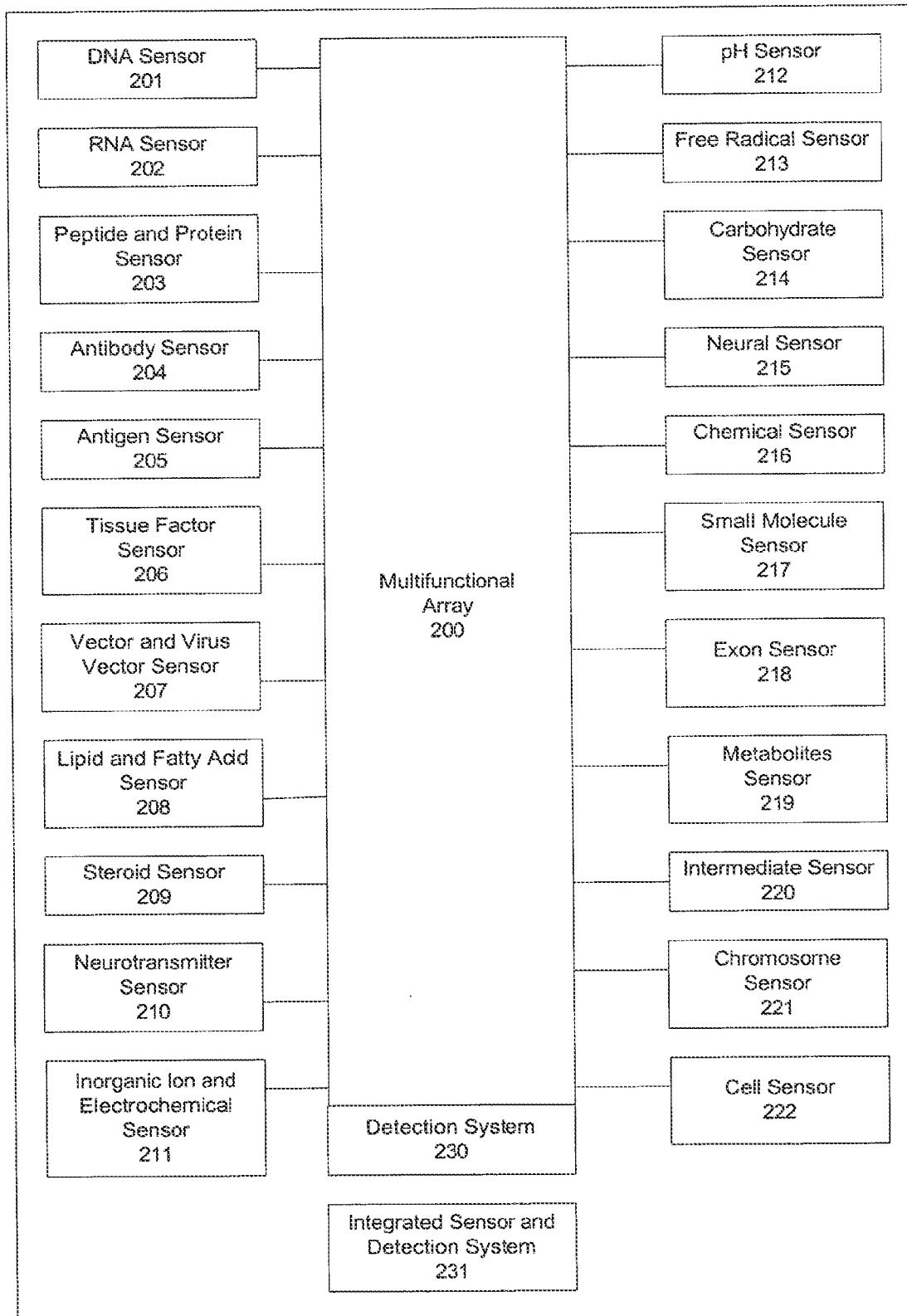
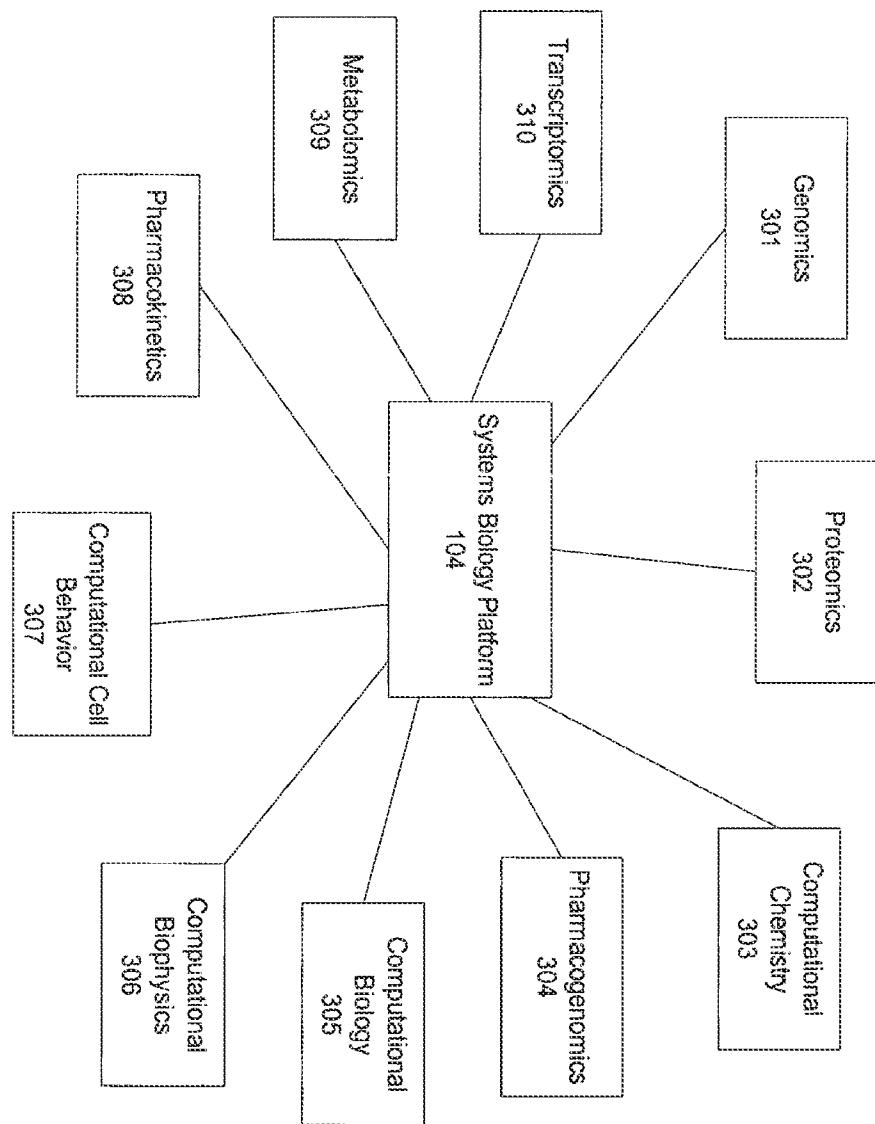


Figure 2

Figure 3a



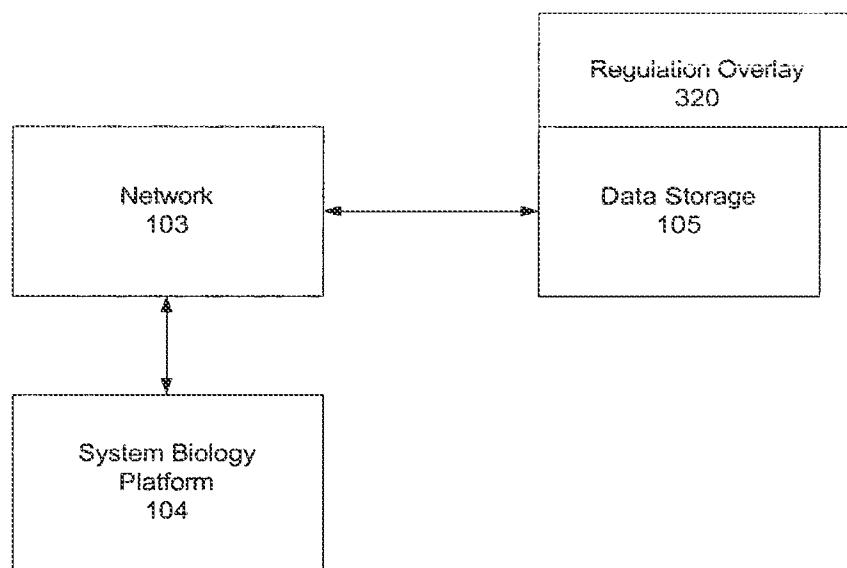


Figure 3b

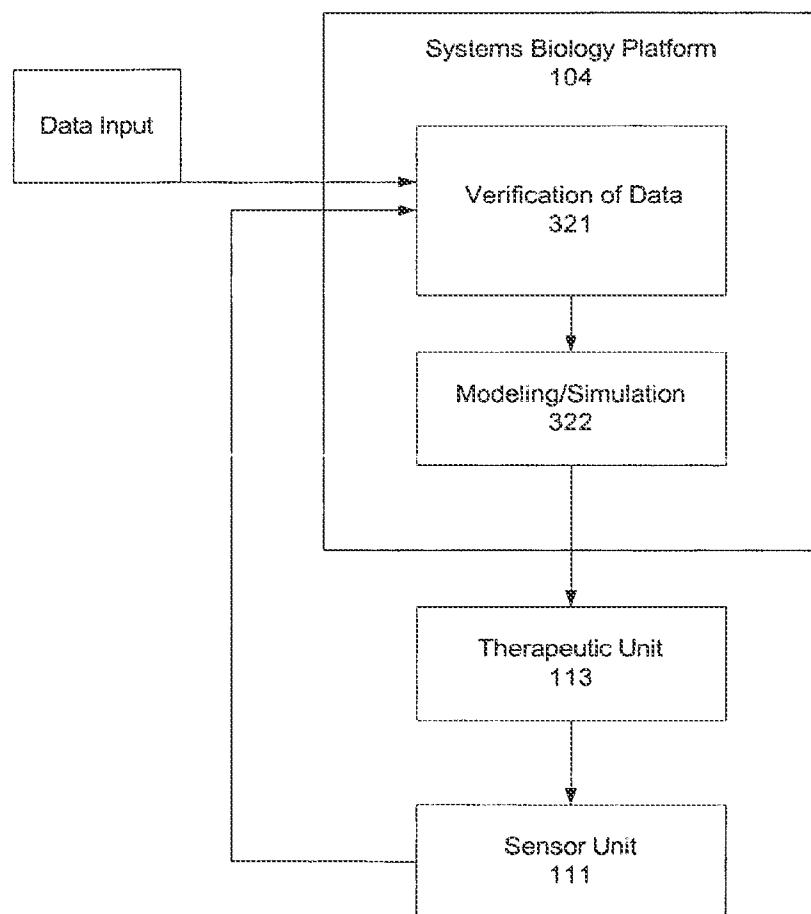


Figure 3c

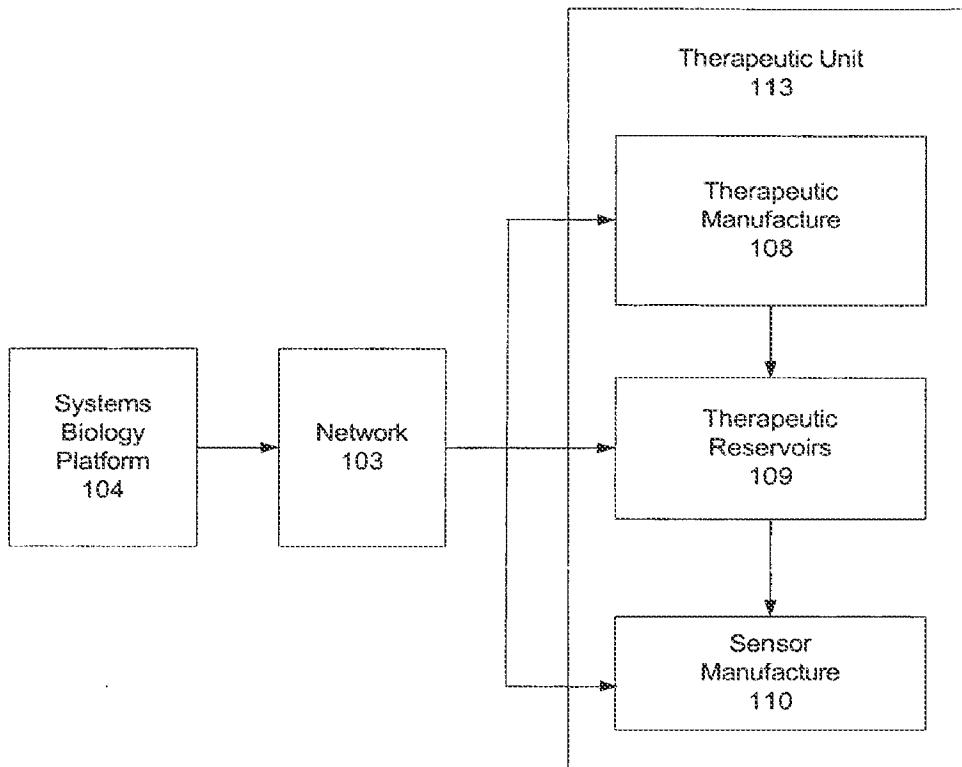


Figure 4a



Therapeutic Manufacture 108

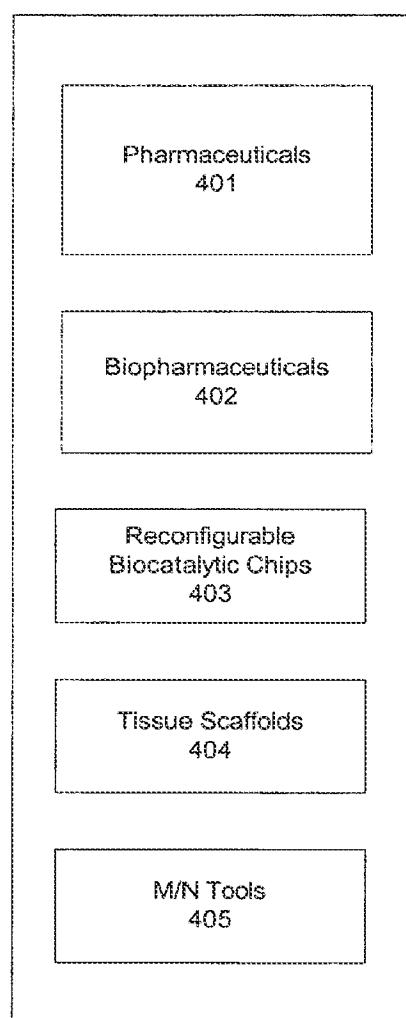


Figure 4b

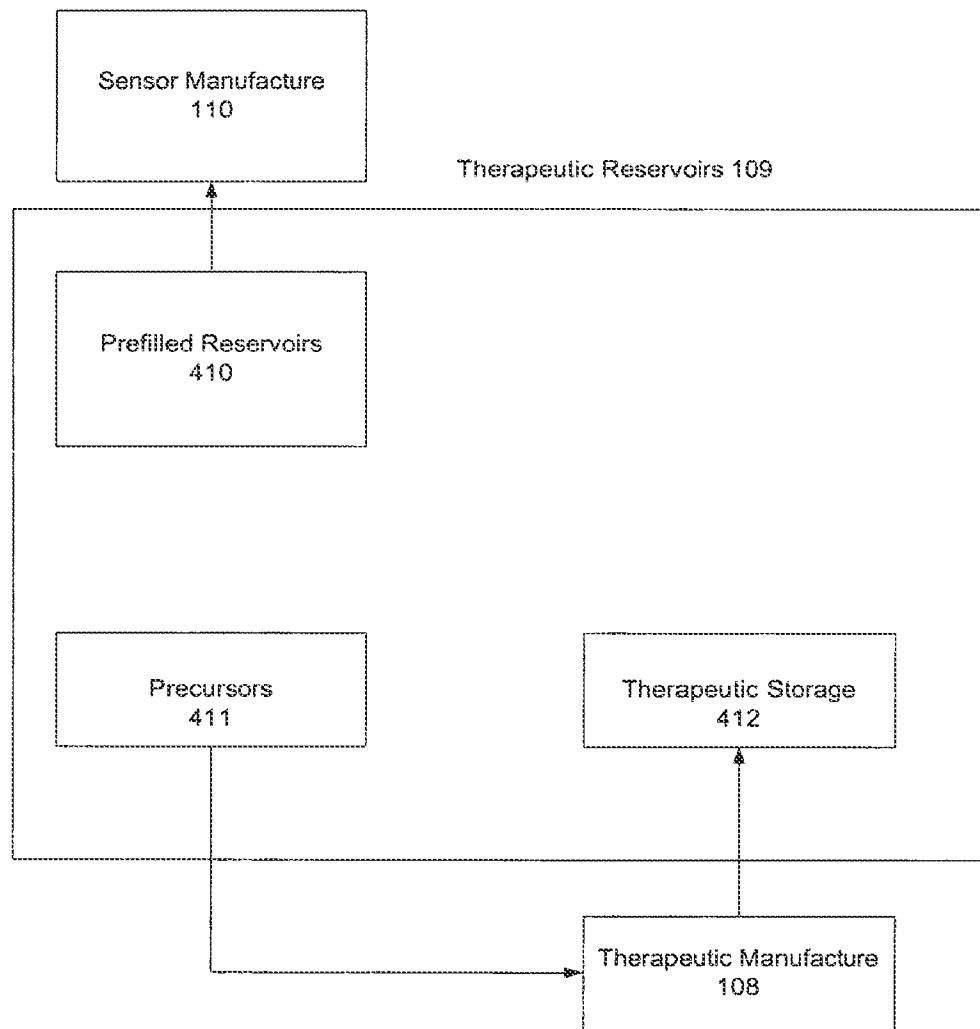


Figure 4c

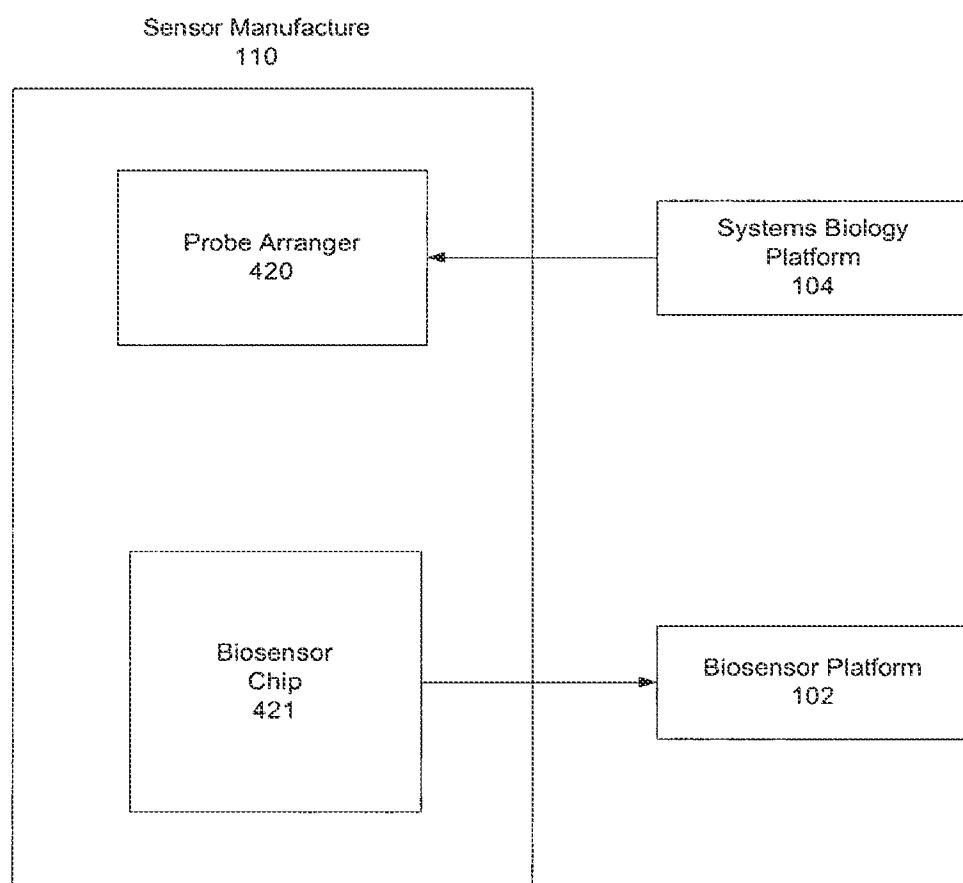


Figure 4d

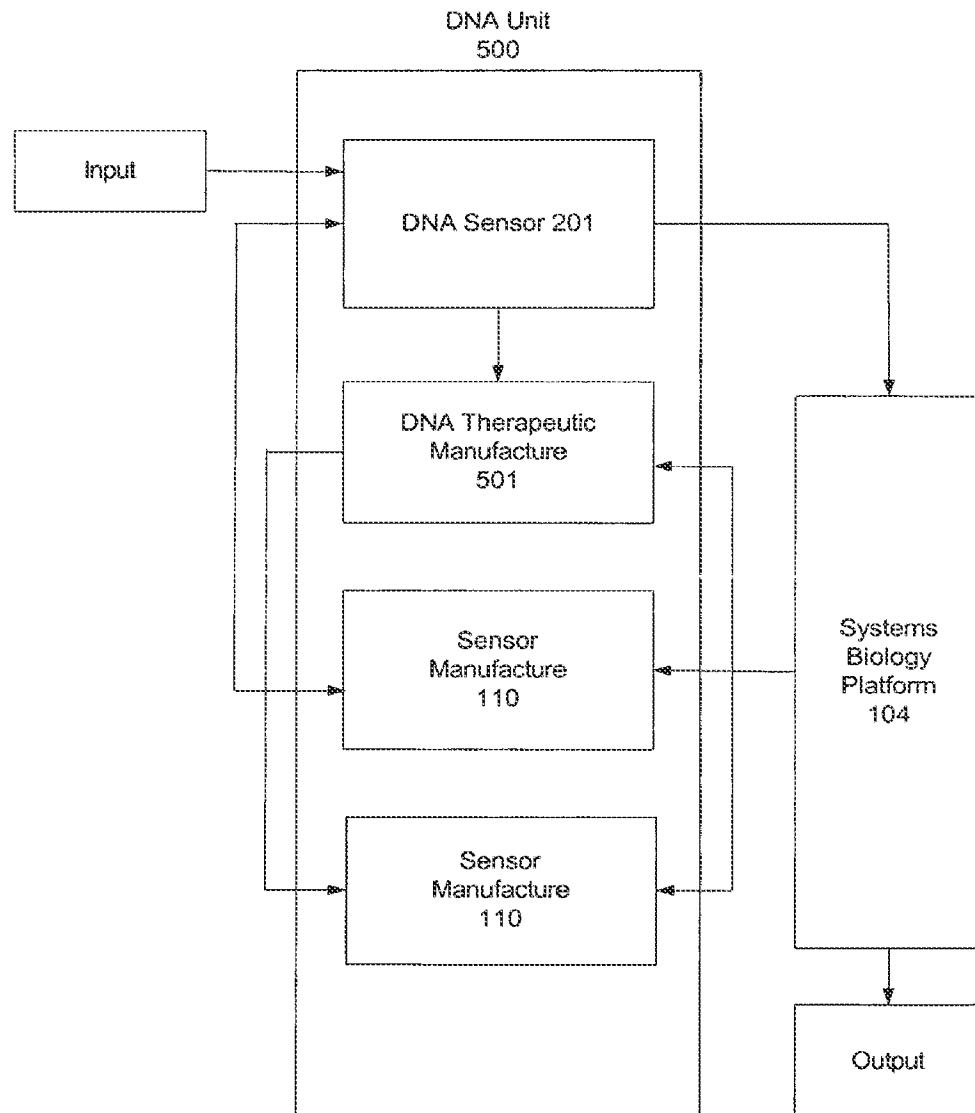


Figure 5

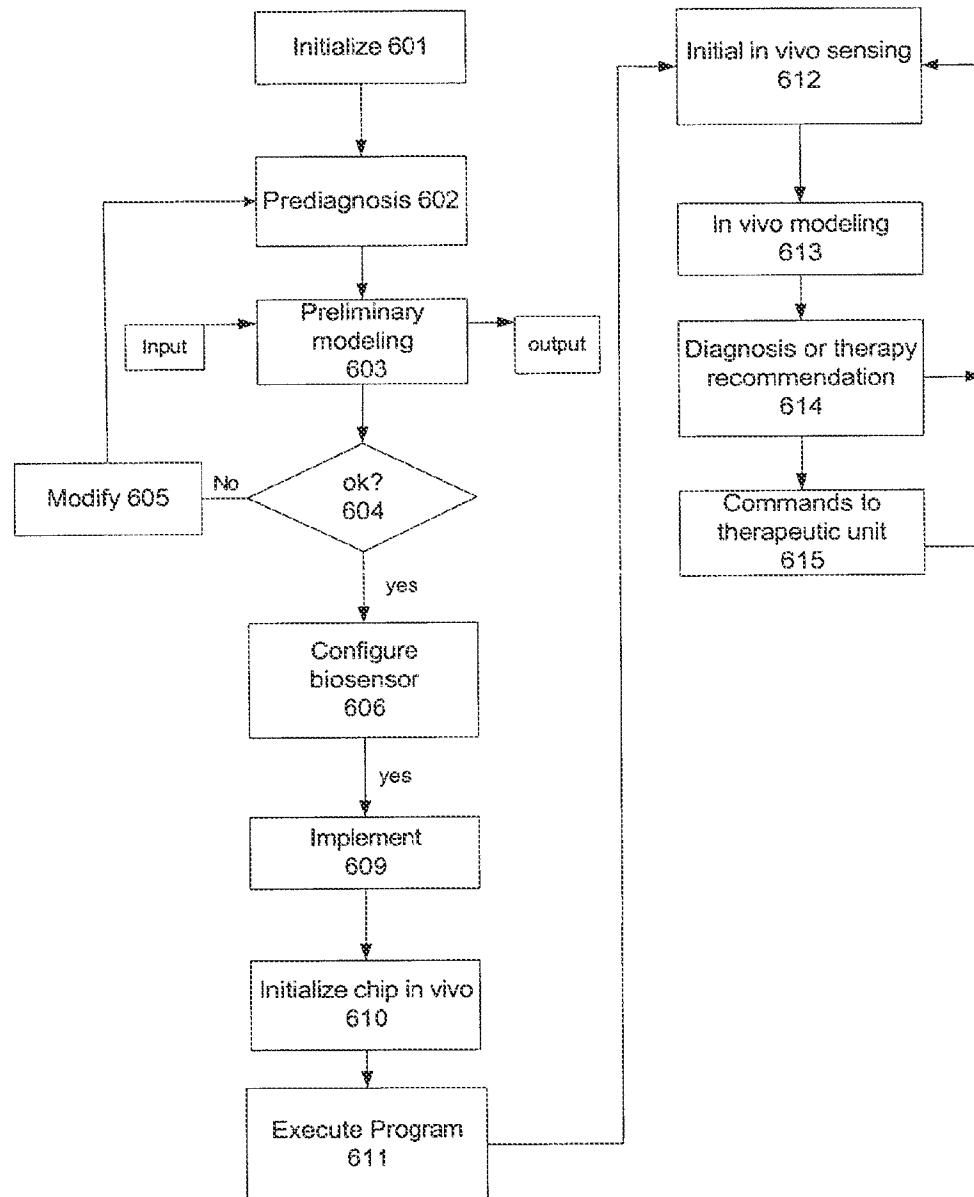


Figure 6



UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/646,682	08/22/2003	Dennis S. Fernandez	FERN-P013	1019
22877	7590	02/03/2010	EXAMINER	
FERNANDEZ & ASSOCIATES, LLP			DEJONG, ERIC S	
P.O. BOX D			ART UNIT	PAPER NUMBER
MENLO PARK, CA 94026			1631	
MAIL DATE		DELIVERY MODE		
02/03/2010		PAPER		

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)
	10/646,682	FERNANDEZ, DENNIS S.
	Examiner	Art Unit
	ERIC S. DEJONG	1631

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) Responsive to communication(s) filed on 05 October 2009.
 2a) This action is FINAL. 2b) This action is non-final.
 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4) Claim(s) 36-55 is/are pending in the application.
 4a) Of the above claim(s) 50 is/are withdrawn from consideration.
 5) Claim(s) _____ is/are allowed.
 6) Claim(s) 36-49 and 51-55 is/are rejected.
 7) Claim(s) _____ is/are objected to.
 8) Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) The specification is objected to by the Examiner.
 10) The drawing(s) filed on _____ is/are: a) accepted or b) objected to by the Examiner.
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
 11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 a) All b) Some * c) None of:
 1. Certified copies of the priority documents have been received.
 2. Certified copies of the priority documents have been received in Application No. _____.
 3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) <input type="checkbox"/> Notice of References Cited (PTO-892)	4) <input type="checkbox"/> Interview Summary (PTO-413)
2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)	Paper No(s)/Mail Date. _____ .
3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____ .	5) <input type="checkbox"/> Notice of Informal Patent Application
	6) <input type="checkbox"/> Other: _____ .

DETAILED OFFICE ACTION

Applicants response filed 10/05/2009 is acknowledged.

Claims 1-35 are cancelled. Claims 36-55 are pending. Claim 50 is withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected species, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 02/15/2008. Claims 36-49 and 51-55 are currently under examination.

Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 36-49 and 51-55 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably

convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection is necessitated by amendments made to the instant claims.

In the instant case, independent claims have been amended so as to recite a systems-biology platform comprising “computational modeling hardware and software genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics” in independent claims 36 and 40. Further, it is noted that applicants did not cite any support for the instant amendment from the instant specification. Upon review of the instant disclosure, the examiner is unable to find any support disclosure in the instant specification for said amendments. Therefore, applicants amendment is considered new matter.

Response to Arguments

Applicant's arguments filed 10/05/2009 have been fully considered but they are not persuasive.

Applicants argument regarding the rejection of claims under 35 USC 112, 2nd indicated Figure 3a as support for “computational modeling hardware and software analysis”, genomics proteomics (paragraph 124), computational chemistry (paragraph 125), pharmacogenomics (paragraph 126), cell behavior (paragraph 127), pharmacokinetics (paragraph 128), metabolomics (paragraph 129), and transcriptomics (paragraph 130), and further descriptions provided on pages 36-38.

In response, it is first noted that applicants have not provided any support for either of the “computational biology” or “computational biophysics” species recited in the instant claim. This alone demonstrates that applicants do not have sufficient written support from the instant specification for the above cited amendment. Further, the support cited by applicant merely is a reiteration of the terms recited in the instant claims does not contain any further description on the actual elements that comprise the “computer modeling hardware and software” as instantly claimed. Such does not amount to any meaningful definition. Thus the instantly claimed limitation of “computer modeling or hardware and software” is undefined and unbounded as it is only limited to broad field of use language and not particular language that identifies actual, meaningful elements that limit the scope of the claimed invention.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 36-49 and 51-55 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The independent claims have been amended so as to recite a systems-biology platform comprising “computational modeling hardware and software analysis genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics,

metabolomics, and transcriptomics" in independent claims 36 and 40. However, this causes the metes and bounds of the instant claims to be indefinite because it cannot be determined what, if any, structural or functional limitations are imposed upon the "systems-biology platform". As amended, the instant claims now recite the nonse terms "computational modeling hardware and software analysis genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics" appears to represent some sort of computational device directed to the generic fields of use specified in the instant claim, specifically the fields of "genomics", "proteomics", "computational chemistry", "pharmacogenomics", "computational biology", "computational biophysics", "computational cell behavior", "pharmacokinetics", "metabolomics", and "transcriptomics". See the precedential BPAI decision *Ex parte Rodriguez* (2009). As indicated in the rejection of claims under 35 U.S.C. 112, 1st for introducing new matter, the instant specification does not describe nor teach any particular computational device that sufficiently describes what the claimed "computational modeling hardware and software analysis genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics" actually comprises as far as components and programming is concerned. Therefore, the instant claims remain indefinite because the scope of the instantly claimed "computational modeling hardware and software" is not defined in either the claims nor the instant specification.

For the purpose of continuing examination and consideration of prior art, the recitation of a systems-biology platform comprising “computational modeling hardware and software genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics” has not been afforded weight because it does not serve to place any meaningful limit on a “systems-biology platform” as instantly claimed.

Response to Arguments

Applicant's arguments filed 10/05/2009 have been fully considered but they are not persuasive.

Applicants argue that the instant claims now recite “computational modeling hardware and software” and therefore the claims are not limited to abstract fields of analysis.

In response, it is reiterated that the support cited by applicant merely is a reiteration of the terms recited in the instant claims does not contain any further description on the actual elements that comprise the “computer modeling hardware and software” as instantly claimed. Such does not amount to any meaningful definition. Thus the instantly claimed limitation of “computer modeling or hardware and software” is undefined and unbounded as it is only limited to broad field of use language and not particular language that identifies actual, meaningful elements that limit the scope of the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 36-49 and 51-55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Porat et al. (US Patent No. 6,432,050) in view of Giuffre (US Patent No. 6,024,548).

The instant claims are drawn to an integrated biosensor and simulation system and method of use. The system comprises an implantable biosensor, and a simulator comprising a systems-biology platform for generating a therapeutic or diagnostic output, wherein said simulator is reconfigurable by said simulator, such reconfiguration thereby reconfiguring a biocatalyst chip, a logic device, a tissue scaffold, a therapeutic reservoir, or a DNA microarray. The related method of use comprises the steps of sensing a biological target to generate a signal, simulating using said signal and a model of the biological target to generate a therapeutic or diagnostic output.

Porat et al. sets forth systems and methods of use for an implantable biosensor system for monitoring and optionally alleviating a physiological condition in a patient (see Porat et al., Abstract and throughout). Porat et al. further teaches the that the implantable biosensor is used to generate a signal comprising information pertaining to a patients physiological condition (see Porat et al., col. 3, lines 35-61). Porat et al. further teaches embodiments wherein the an implantable biosensor system comprising

a shunt a having fluid passageway and being operable for draining fluid through a fluid passage way from a portion of the patient body (see Porat et al., col. 3, line 62 through col. 4, line 19), which reads on a reconfigurable sensor, wherein reconfiguration involves reconfiguring a therapeutic reservoir as instantly claimed.

While Porat et al. teaches the activation of the above described implantable, reconfigurable biosensor involving a shunt having a fluid passageway, Porat et al. teaches that the activation of said shunt is based on monitored physiological conditions. Porat et al. does not expressly teach the use of a simulator comprising a system-biology platform and a model to generate a therapeutic or diagnostic output, whereby a biosensor is reconfigured by a simulator.

Giuffre discloses a method and a system for registering changes in brain and central nervous system activity by using simulation and signals derived from biosensors (e.g., cardiovascular signal) (See Giuffre, Abstract, col. 4, lines 6-17, and claims 1, 5, 7, 8, 12, and 18). Giuffre discloses generating a signal of a biological target by a biosensor (col. 9, lines 26-37), which reads on a sensor, as recited in claims 36 and 40, and the process step of sensing a biological target to generate a signal, as recited in claim 40. Giuffre discloses a programmable computer systems for simulation of brain activity using a signal data and a model to estimate brain and central nervous system activity (see Giuffre, col. 4, line 6 through col. 5, line 11), which reads on a simulation comprising a system-biology platform, as recited in claims 36 and 40, and the process step of simulating using the signal and a model of the target to generate a therapeutic or diagnostic output, as recited in claim 40. Further, the instant specification is relied upon

for determining the scope of a "systems-biology platform" (see page 36, line 16-20), as a system that uses software for analyzing computational behavior of a biological system. Giuffre discloses embodiments of trained neural net and self-teaching computer systems that act in real-time to incrementally perturb a system and/or change models until data management is optimal (see Giuffre, Fig 3., col. 4, lines 6-60 and col. 6, lines 53-59), which reads on a sensor reconfigurable by a simulator, as recited in claims 36 and 40, and the process step of a simulator reconfiguring a sensor, as recited in claim 40.

Giuffre further teaches the detection of drug infusions and drug and alcohol levels in the blood for use in the disclosed method and a system for registering changes in brain and central nervous system activity (see Giuffre, col. 7, line 44 through col. 8, line 2), which reads on a sensor that senses a food material for consumption by a biological target, the generation of a second signal therefrom, and the use of said second signal to generate a therapeutic or diagnostic output as recited claims 37 and 41. Giuffre teaches generating an output according to a regulatory condition by the disclosed simulation system (see Giuffre, col. 7, line 44 through col. 8, line 24), as recited in claims 38 and 42. Giuffre discloses coupling using a trained neural net and self-teaching computer systems (a switch) (see Giuffre, Figs. 1-3 and col. 4, lines 6-60), which reads on a sensor coupled to a simulator via a programmable switch as recited in claims 39 and 43.

Giuffre further teaches the use of separate biosensors for the heart and brain (see Giuffre, col. 4, lines 6-38), which reads on the implantation of a biosensor for the

heart and brain, as recited in claims 44 and 52, an array of at least two sensors capable of sensing two different biological targets, as recited in claims 45, 46, 49, 53, and 54, and a neural biological target, as recited in claims 47 and 55. Giuffre further teaches that the disclosed method relies upon neurophysiological and cardiovascular monitoring from said biosensors for training a neural network (see Giuffre, col. 3, lines 55-61 and col. 4, lines 6-60). Following the training of a neural network, Giuffre further teaches that only cardiovascular monitoring by heart associated biosensor and the trained neural network are relied upon to estimate the neurophysiological state of a patient (see Giuffre, col. 4, lines 17-38), which reads on the elected species of reconfiguration comprising activating or deactivating at least one biosensor, as recited in claims 48, 49, 51, and 54.

Therefore it would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains to combine the biosensor system and methods, set forth by Porat et al., in combination with the method and a system for registering changes in brain and central nervous system activity by using simulation and signals derived from biosensors, as taught by Giuffre. One of ordinary skill in the art would further be motivated to combine the systems and methods set forth by Porat et al. with that of Giuffre because Giuffre teaches that systems that can predict brain states using already implemented cardiovascular monitoring modalities will allow for predictive capabilities while minimizing risk, cost, and added complexity of such setups (see Giuffre, col. 1, lines 5-25).

Response to Arguments

Applicant's arguments filed 10/05/2009 have been fully considered but they are not persuasive.

In regards to the rejection of claims under 35 USC 103(a) as being unpatentable over Porat et al. in view of Giuffre, applicants argue that the instant claims have been amended to recite a system-biology platform further comprising "computer modeling hardware and software analysis genomics, proteomics, computational chemistry, pharmacogenomics, computational biology, computational biophysics, computational cell behavior, pharmacokinetics, metabolomics, and transcriptomics" to further distinguish the instant claims over the applied prior art of Giuffre.

In response, the recited series of scientific disciplines has not been afforded patentable weight with regard to consideration of the prior art because they do not serve to modify or place any apparent limits on the "systems-biology platform" as instantly claims. See also the above rejections under 35 USC 112, 1st and 2nd paragraphs. Therefore, applicants argument is not persuasive as the systems-biology platform recited in the instant claims is not distinguished over the prior art of record.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ERIC S. DEJONG whose telephone number is (571)272-6099. The examiner can normally be reached on 8:30AM-5:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Marjorie Moran can be reached on (571) 272-0720. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR.

Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ERIC S. DEJONG/
Primary Examiner, Art Unit 1631

NIH WORKING DEFINITION OF BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

July 17, 2000

The following working definition of bioinformatics and computational biology were developed by the BISTIC Definition Committee and released on July 17, 2000. The committee was chaired by Dr. Michael Huerta of the National Institute of Mental Health and consisted of the following members:

Bioinformatics Definition Committee

BISTIC Members

Michael Huerta (Chair)
Florence Haseltine
Yuan Liu

Expert Members

Gregory Downing
Belinda Seto

Preamble

Bioinformatics and computational biology are rooted in life sciences as well as computer and information sciences and technologies. Both of these interdisciplinary approaches draw from specific disciplines such as mathematics, physics, computer science and engineering, biology, and behavioral science. Bioinformatics and computational biology each maintain close interactions with life sciences to realize their full potential.

Bioinformatics applies principles of information sciences and technologies to make the vast, diverse, and complex life sciences data more understandable and useful.

Computational biology uses mathematical and computational approaches to address theoretical and experimental questions in biology. Although bioinformatics and computational biology are distinct, there is also significant overlap and activity at their interface.

Definition

The NIH Biomedical Information Science and Technology Initiative Consortium agreed on the following definitions of bioinformatics and computational biology recognizing that no definition could completely eliminate overlap with other activities or preclude variations in interpretation by different individuals and organizations.

Bioinformatics: Research, development, or application of computational tools and approaches for expanding the use of biological, medical, behavioral or health data, including those to acquire, store, organize, archive, analyze, or visualize such data.

Computational Biology: The development and application of data-analytical and theoretical methods, mathematical modeling and computational simulation techniques to the study of biological, behavioral, and social systems.

Application of John H. WERTHEIM
et al.

Patent Appeal No. 75-536.

United States Court of Customs,
and Patent Appeals.

Aug. 26, 1976.

Applicant for patent serial No. 96,286, with respect to a process for making freeze-dried instant coffee appealed from a decision of the Patent and Trademark Office Board of Appeals affirming the rejection of all claims in application. The appeal as to certain claims was withdrawn. The Court of Customs and Patent Appeals, Rich, J., held that certain claims were entitled to the benefit of the earlier filing date of Swiss application of applicants and were therefore improperly rejected while certain other claims were not entitled to such earlier filing date and were properly rejected and that certain other claims were properly rejected on ground of obviousness in view of prior art while other claims were improperly rejected on grounds of obviousness.

Affirmed in part and reversed in part.

Baldwin, J., filed an opinion concurring in part and dissenting in part.

Miller, J., filed an opinion dissenting in part and concurring in part.

1. Patents \Leftrightarrow 90(1)

If patent applicants' parent and Swiss applications complied with specification statute, including description requirement, as to the subject matter of the interference claims, the claims were entitled to filing dates of parent application and Swiss application. 35 U.S.C.A. §§ 112, 119, 120.

2. Patents \Leftrightarrow 101(5)

Function of description requirement with respect to application is to ensure that inventor had possession, as of filing date of application relied on, of specific subject matter later claimed by him; how specification accomplishes this is not material. 35 U.S.C.A. § 112.

3. Patents \Leftrightarrow 101(5)

It is not necessary that application for patent describe claim limitations exactly but only so clearly that persons of ordinary skill in the art will recognize from disclosure that applicants invented processes including those limitations. 35 U.S.C.A. § 112.

4. Patents \Leftrightarrow 101(5)

In determining compliance with description requirement of statute with respect to limitations, the primary consideration is factual and depends on nature of invention and amount of knowledge imparted to those skilled in the art by the disclosure. 35 U.S.C.A. § 112.

5. Patents \Leftrightarrow 113(7)

On appeal from decision of patent and trademark office board of appeals affirming final rejection of claim, PTO had initial burden of presenting evidence of reasons that persons skilled in art would not have recognized in disclosure a description of invention defined by claims, and by pointing to fact that the claim read on embodiments outside scope of description the PTO satisfied its burden. 35 U.S.C.A. § 112.

6. Patents \Leftrightarrow 101(2)

Where Swiss application on which applicant for continuation patent relied was filed prior to issuance of United States patent, for purpose of statute relating to specifications in patent application the United States patent disclosure was not evidence of what those skilled in art considered conventional at the time Swiss application was filed. 35 U.S.C.A. § 112.

7. Patents \Leftrightarrow 90(1)

Claims 1 and 4 of application relating to method for making freeze-dried instant coffee were not entitled to benefit of filing date of applicants' earlier Swiss application for patent since the claims in instant application relied on embodiments employing solids content outside range described in Swiss application.

8. Patents \Leftrightarrow 101(5)

Where it is clear that the broad described range pertains to a different inven-

tion than narrower claimed range, then broader range does not describe narrower range for purpose of statute relating to specifications in application for patent. 35 U.S.C.A. § 112.

9. Patents == 66(124), 90(1)

Claims 2, 37 and 38 of patent application relating to process for making freeze-dried instant coffee claiming a solids content range within the described broad range of Swiss application were entitled to benefit of the filing date of the Swiss application which antedated the United States patent, which was not available as a prior art of its 1968 date, so that rejection of such claims was improper. 35 U.S.C.A. §§ 102(e), 103, 112.

10. Patents == 101(11)

Claims 6-10, 12-15, 17, and 26 relating to application for patent for making freeze-dried instant coffee were improperly rejected on ground that limitation of particle size was not described in application as originally filed and was added to the application in violation of statute, since the originally filed specification clearly conveyed to those of ordinary skill in art that applicants invented process in which the particles were of particular size. 35 U.S.C.A. § 132.

11. Patents == 51(1)

Disclosure in prior art of any value within a claimed range is an anticipation of the claimed range. 35 U.S.C.A. § 103.

12. Patents == 18

With respect to patent application relating to process for making freeze-dried instant coffee, claims 6-14, 16, and 21-28 were properly rejected on ground of obviousness in view of the prior art while process claims 17-20 and 29 were improperly rejected on grounds of obviousness. 35 U.S.C.A. § 103.

13. Patents == 18

Apparatus claims 30-35 of application for patent, with respect to a process for

1. A continuation (or continuation-in-part, as the examiner has required it to be denominated) of application serial No. 537,679, filed March 28, 1968. Appellants claim the benefit of a Swiss application filed April 2, 1965. The

making freeze-dried instant coffee were properly rejected on grounds of obviousness in view of prior art. 35 U.S.C.A. § 103.

14. Patents == 18

Patent claims 15 and 40-43 of application for patent relating to process for making freeze-dried instant coffee were properly rejected for obviousness in view of prior art.

William H. Vogt, III, Watson, Leavenworth, Kelton & Taggart, New York City, attys. of record, for appellants; Paul E. O'Donnell, Jr., New York City, of counsel.

Joseph F. Nakamura, Washington, D. C., for the Commissioner of Patents; Gerald H. Bjorge, Washington, D. C., of counsel.

Before MARKEY, Chief Judge, and RICH, BALDWIN, LANE and MILLER, Judges.

RICH, Judge.

This appeal is from the decision of the Patent and Trademark Office (PTO) Board of Appeals affirming the final rejection of claims 1-43, all the claims in application serial No. 96,285, filed December 8, 1970, entitled "Drying Method."¹ The appeal on claims 8, 5, 36, and 39 has been withdrawn, and as to these claims it is, therefore, dismissed. As to the remaining claims, we affirm in part and reverse in part.

The Invention

Appellants' invention centers around a process for making freeze-dried instant coffee. Claims 1, 6, 80, and 40 are illustrative:

1. An improved process for minimizing loss of volatiles during freeze-drying of coffee extract which comprises obtaining coffee extract, concentrating said extract to a higher solids level of at least 35%, foaming said concentrated extract

title of the application on appeal is somewhat inaccurate, as the application contains claims to apparatus for drying and dried instant coffee products as well as to a drying method.

to a substantial overrun by injection of a gas into said extract at at least atmospheric pressure to thereby avoid evaporative cooling due to evaporation of water in said extract during said foaming, freezing said foam to below its eutectic point at at least atmospheric pressure while avoiding evaporative cooling, and freeze-drying said extract at below the eutectic temperature of said extract.

6. Process for preparing a powdered coffee extract, which comprises adding sufficient inert gas to a concentrated aqueous extract of roast coffee containing about 25% to 60% by weight of soluble coffee solids to provide a foam having a density between about 0.4 and 0.8 gm/cc, freezing the foamed extract to a solid mass, grinding the frozen foam to a particle size of at least 0.25 mm and freeze drying the ground frozen foam.

30. An apparatus for carrying out the process defined in claim 6 comprising, in combination, means for foaming, a closed chamber capable of being maintained at a temperature which is substantially below the melting temperature of said frozen foam, and, disposed within said chamber, a movable endless belt, means for moving said belt at a low speed, a spreading device for distributing coffee extract foam on said belt and refrigerating means for cooling at least one surface of said belt with a liquid refrigerant.

40. A dry coffee powder comprising a freeze-dried particulated foamed extract of roast and ground coffee, the foam before freeze drying having a density between about 0.4 and 0.8 gm/cc.

The remaining claims are reproduced in the Appendix hereto. Appellants assert that their invention produces an instant coffee having a bulk density of 0.2-0.3 gm/cc, which corresponds to that of conventional spray-dried instant coffee.² They allege they discovered that this desired bulk density

results from controlling the solids content of the concentrated extract prior to foaming and the density of the foam generated therefrom within the ranges of their freeze-drying process claims.

Since the claims are somewhat elliptical in setting out the steps of appellants' process, we shall describe it further. An aqueous extract of coffee is prepared by percolating hot water through roasted and ground coffee beans. The extract is concentrated to have a solids content between 25% and 60% and is then charged with gas to produce a foam having a density between 0.4 and 0.8 gm/cc. The foam is frozen and ground into particles, preferably 0.25 to 2.0 mm in size, which are freeze-dried by conventional techniques.

Prosecution History and Rejections

The claims which remain on appeal fall into two broad groups: The "interference" claims 1, 2, 4, 37, and 38; and the "non-interference" claims, 6-35 and 40-43.

As originally filed, the application contained claims 1-5 copied from Pfluger et al. U. S. Patent No. 3,482,990 (Pfluger patent), issued December 9, 1969, on an application filed February 19, 1969. A letter under Rule 205(a), 37 CFR 1.205(a), requesting an interference with the Pfluger patent accompanied the application. By amendment, appellants transferred claims 6-35 from their 1966 application to the instant application. Claims 36-39, added by amendment, are modified versions of the previously copied claims and were presented for the purpose of providing a basis for phantom counts in an interference with the Pfluger patent under Rule 205(a) and Manual of Patent Examining Procedure § 1101.02. They depend from claim 2.

2. So that consumers may continue to use the same amount of freeze-dried instant coffee per cup as conventional instant coffee without

change in the strength of the beverage that they are accustomed to.

The patents relied on by the examiner are:

Pfluger et al.	3,482,990	Dec. 9, 1969
De George	3,283,420	May 31, 1966
(application filed Feb. 3, 1966)		
Carpenter et al.	2,974,497	Mar. 14, 1961
British patent	948,517	Feb. 5, 1964

The Pfluger patent issued on a chain of four applications: serial No. 800,853, filed Feb. 10, 1969, which was a continuation of serial No. 520,347, filed Jan. 18, 1966 (Pfluger 1966), which was a continuation-in-part of serial No. 309,410, filed Sept. 17, 1962 (Pfluger 1963), which was a continuation-in-part of serial No. 98,007, filed Mar. 24, 1961. The Pfluger patent discloses a process for making freeze-dried instant coffee which has as its goal minimizing the loss from a foamed extract of volatile aromatics which contribute substantially to the natural flavor of coffee and other foods.

De George describes apparatus and methods for freezing liquid, unfoamed coffee extract prior to drying on continuous belts refrigerated by brine tanks contacting the bottom surfaces of the belts. The claims of De George are directed to processes for facilitating the removal of the frozen sheet of coffee extract from the belt before it is freeze dried.

The British patent discloses a rapid freeze-drying process in which the food product is frozen, milled into small particles which are spread from a hopper in single-particle layers onto plates, and freeze-dried in a vacuum chamber. More details of the disclosure are supplied *infra*.

Carpenter discloses the cooling of a refrigeration belt by spraying cold brine onto the underside of the belt.

3. 37 CFR 1.204(c):

When the effective filing date of an applicant is more than 3 months subsequent to the effective filing date of the patentee, the applicant, before the interference will be declared, shall file two copies of affidavits or declarations by himself, if possible, and by one or more corroborating witnesses, supported by documentary evidence if available, each setting out a factual description of acts and circumstances performed or observed by the affiant, which collectively would prima

The examiner made multiple rejections which were addressed by the board in eight categories, seven of which are before us for review. Category 1 covers the "interference" claims, which were rejected on the Pfluger patent, claims 1, 2, and 4 under 35 U.S.C. § 102 and claims 37 and 38 under § 103. The board agreed with the examiner's position that these claims were not entitled to the benefit of appellants' 1966 Swiss priority date because they were not supported by appellants' parent and Swiss applications. The limitations held to be unsupported were "at least 35% [solids content]" in claim 1, "between 35% and 60% soluble solids" in claims 2 and 4, and "pressure of less than 500 microns" and "final product temperature of less than 110°F." in claim 4. For that reason appellants were held to be junior to the Pfluger patent on the basis of Pfluger's 1966 filing date. In light of appellants' refusal to file a Rule 204(e)² affidavit showing a date of invention prior to Pfluger's 1966 filing date, the examiner and the board held the Pfluger patent to be prior art under § 102(e) against claims 1, 2, 4, 37, and 38 and rejected the claims on that basis.⁴ The board refused to hold that the claims were supported in the parent and Swiss applications, "for interference purposes," under our decision in *In re Waymouth*, 486 F.2d 1058, 179 USPQ 627 (Cust. & Pat.App.1973), *mod. on reh.*, 489 F.2d 1297, 180 USPQ 483 (CCPA 1974). The board stated that appellants' failure to file a Rule 204(e) affidavit precluded any attempt to get into an interference and that *Waymouth*, which concerned the right to make a claim for interference purposes in the application on appeal, was therefore inapplicable to this case.

facis entitle him to an award of priority with respect to the effective filing date of the patent. This showing must be accompanied by an explanation of the basis on which he believes that the facts set forth would overcome the effective filing date of the patent.

4. The examiner and the board did not rely on Pfluger 1963 because the solids content and foam density ranges of the copied claims were not described in that application. *In re Lund*, 376 F.2d 982, 54 CCPA 1361, 153 USPQ 625 (1967).

Under Category II, the board affirmed the rejection of claims 6-10, 12-15, 17, and 26 under 35 U.S.C. § 102 for new matter. The board held that these claims, which were added to the instant application by amendment, were not supported in the original disclosure for lack of a description of the claimed size of the ground foam particles, i. e., "at least 0.25 mm."

The Category III rejection was reversed by the board.

In Category IV, claims 6-8, 11-20, and 40-43 were rejected under § 103 on the disclosure of Pfluger 1963⁵ carried forward to the Pfluger patent, in accordance with *In re Lusd*, *supra*. The board found that the foam density range of 0.4-0.8 gm/cc claimed by appellants (and the 0.6-0.8 gm/cc range in claims 19 and 20) was suggested by Pfluger 1963's disclosure of 0.1-0.5 gm/cc foam density and that Pfluger 1963 teaches the use of foaming gases and concentrating the coffee extract prior to foaming. The board found that the final product densities claimed would be inherent "in view of the same foam overrun density disclosed by Pfluger" and that Pfluger's example I, which discloses breaking the frozen foam strands into 1/4" lengths (i. e., "at least 0.25 mm") before drying, would suggest the size of the ground foam particles claimed by appellants.

Category V added De George to the § 103 rejection of claims 9, 10, 30, and 32-35. The board agreed with the examiner that the temperatures, foam thicknesses, and belt lengths and speeds covered by these claims are disclosed in De George, and that it would be obvious to use De George's moving belt apparatus in the Pfluger process.

In Category VI claims 21-23 and 26-29 were rejected under § 103 on Pfluger in view of the British patent, which was relied on for its teaching of the concentration of coffee extract by freezing to a solids content of 27 to 28%. Pfluger was applied to

5. Peebles U. S. patent No. 2,897,084, issued July 28, 1962, was cited against claims 19 and 26 to show that agglomerating fine dried coffee particles into larger grounds was old in the art.

the claims under the rationale employed in Category IV.

Category VII was the rejection of claims 24 and 25 under § 103 on Pfluger, the British patent, and De George, which was relied on to show "the deposition of a coffee extract on a moving belt prior to grinding and freeze drying." The board otherwise relied on the reasoning in Categories V and VI.

Under Category VIII claim 31 was rejected on Pfluger and De George under § 103 for the reasons of Category V, with reliance on Carpenier to show refrigeration of the belt by spraying refrigerant onto the bottom of the belt instead of using De George's brine tanks.

OPINION

The "Interference" Claims—1, 2, 4, 37, and 38

(1) The dispositive issue under this heading is whether appellants' parent and Swiss applications comply with 35 U.S.C. § 112, first paragraph, including the description requirement, as to the subject matter of these claims. If they do, these claims are entitled to the filing dates of the parent application under 35 U.S.C. § 120; *In re Lakach*, 442 F.2d 967, 58 CCPA 1233, 189 USPQ 795 (1971), and the Swiss application under 35 U.S.C. § 119, *Kawai v. Mettlerics*, 480 F.2d 890, 887-88, 178 USPQ 158, 164 (Cust. & Pat.App.1978). Since the PTO relies only on Pfluger 1966 to provide the effective U.S. filing date of the patent as a reference against these claims under §§ 102(e) and 103, a right of foreign priority in appellants' Swiss application will anticipate Pfluger 1966 and remove it as prior art against the claims.

The only defect asserted below in appellants' parent and Swiss application disclosures that covers all these claims is that the applications do not contain written descriptions of the solids content limitations of the

Appellants have acknowledged this to be true, so it is not necessary to discuss Peebles further.

concentrated extract prior to foaming, i. e., "at least 35%" (claim 1) and "between 35% and 60%" (claims 2, 4, 27, and 28).⁶

Appellants' parent and Swiss applications contain virtually identical disclosures on this point. Both disclose that the coffee extract initially produced by percolation of water through ground roasted coffee is concentrated prior to foaming by suitable means "until a concentration of 25 to 60% solid matter is reached." Examples in each disclose specific embodiments having solids contents of 36% and 50%.

In our view, it is necessary to decide only whether the Swiss application complies with the description requirement of § 112 with respect to the questioned limitations. There is no question that the instant application supports claims 1, 2, and 4, which are original claims in that application. Appellants and the solicitor urge us to decide this case by determining whether the broad rule of *In re Waymouth*, *supra*, is still valid or must be disapproved. In the interest of judicial economy, we decline this entreaty since the issue of whether the Swiss application contains written descriptions of the disputed limitations of claims 1, 2, 4, 27, and 28, being addressed to strict compliance with § 112, first paragraph, is dispositive regardless of the validity of Waymouth in its own factual setting. The sufficiency of the parent U.S. application need not be separately decided since appellants must have the benefit of their Swiss application date to antedate the Pfluger patent.

[2, 3] The function of the description requirement is to ensure that the inventor had possession, as of the filing date of the application relied on, of the specific subject matter later claimed by him; how the specification accomplishes this is not material.

6. The solicitor belatedly asserts that the Swiss application is not "for the same invention" as the parent application, insofar as claims 1, 2, and 4 are concerned; he argues that the expression "same invention" in 35 U.S.C. § 119 should be given the meaning employed by us in the double patenting cases, e. g., *In re Vogel*, 422 F.2d 438, 57 CCPA 920, 164 USPQ 619 (1970). As we indicated in *In re Ziegler*, 347 F.2d 642, 52 CCPA 1473, 146 USPQ 76 (1965),

In re Smith, 481 F.2d 910, 178 USPQ 620 (Cust. & Pat.App.1973), and cases cited therein. It is not necessary that the application describe the claim limitations exactly, *In re Lukach*, *supra*, but only so clearly that persons of ordinary skill in the art will recognize from the disclosure that appellants invented processes including those limitations. *In re Smythe*, 480 F.2d 1378, 1382, 178 USPQ 279, 284 (Cust. & Pat.App. 1973).

[4] The primary consideration is *factual* and depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure. The factual nature of the inquiry was emphasized in *In re Ruschig*, 379 F.2d 990, 995-96, 54 CCPA 1551, 1558-59, 154 USPQ 118, 123 (1967), which involved the question whether a broad generic disclosure "described" the single chemical compound claimed:

But looking at the problem, as we must, from the standpoint of one with no foreknowledge of the specific compound, it is our considered opinion that the board was correct in saying:

Not having been specifically named or mentioned in any manner, one is left to selection from the myriads of possibilities encompassed by the broad disclosure, with no guide indicating or directing that this particular selection should be made rather than any of the many others which could also be made.

Appellants refer to 35 U.S.C. § 112 as the presumed basis for this rejection and emphasize language therein about enabling one skilled in the art to make the invention, arguing therefrom that one skilled in the art would be enabled by the specification to make chlorpropamide. We find the argument unpersuasive for two reasons. First, it presumes some mo-

the solicitor's reading is too narrow. All § 119 requires is that the foreign application describe and seek protection for "broadly the same invention" as described in the U.S. application claiming its benefit. 347 F.2d at 649, 52 CCPA at 1481, 146 USPQ at 82. The Swiss application has essentially the same disclosure as appellants' parent application and claims broadly the same invention.

tivation for wanting to make the compound in preference to others. While we have no doubt a person so motivated would be enabled by the specification to make it, this is beside the point for the question is not whether he would be so enabled but whether the specification discloses the compound to him, specifically, as something appellants actually invented. We think it does not. Second, we doubt that the rejection is truly based on section 112, at least on the parts relied on by appellants. If based on section 112, it is on the requirement thereof that "The specification shall contain a written description of the invention * * *." [Emphasis ours.] We have a specification which describes appellants' invention. The issue here is in no wise a question of its compliance with section 112, it is a question of fact: *Is the compound of claim 18 described therein?* Does the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound?

Broadly articulated rules are particularly inappropriate in this area. See, e. g., *In re Smith*, 458 F.2d 1389, 1394, 59 CCPA 1023, 1028, 173 USPQ 679, 683 (1972), in which this court felt obliged to overrule a supposed "rule" of *In re Kisse*, 378 F.2d 948, 952-53, 54 CCPA 1496, 1500-01, 154 USPQ 1, 5 (1967). Mere comparison of ranges is not enough, nor are mechanical rules a substitute for an analysis of each case on its facts to determine whether an application conveys to those skilled in the art the information that the applicant invented the subject matter of the claims. In other words, we must decide whether the invention appellants seek to protect by their claims is part of the invention that appellants have described as theirs in the specification. That what appellants claim as patentable to them is less than what they describe as their invention is not conclusive if their specification also reasonably describes that which they do claim. Inventions are constantly made which turn out not to be patentable, and applicants frequently discover during the course of prosecution that only a

part of what they invented and originally claimed is patentable. As we said in a different context in *In re Saunders*, 444 F.2d 599, 607, 58 CCPA 1316, 1327, 170 USPQ 213, 220 (1971):

To rule otherwise would let form triumph over substance, substantially eliminating the right of an applicant to retreat to an otherwise patentable species merely because he erroneously thought he was first with the genus when he filed. Cf. *In re Ruff*, 256 F.2d 590, 597, 45 CCPA 1037, 1049, [118 USPQ 340, 347] (1958). Since the patent law provides for the amendment during prosecution of claims, as well as the specification supporting claims, 35 USC 132, it is clear that the reference to "particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention" in the second paragraph of 35 USC 112 does not prohibit the applicant from changing what he "regards as his invention" (i. e., the subject matter on which he seeks patent protection) during the pendency of his application. Cf. *In re Brower*, 432 F.2d 812, 817, 58 CCPA 724, [728], [167 USPQ 684, 687] (1970) (fact that claims in continuation application were directed to subject matter which appellants had not regarded as part of their invention when the parent application was filed held not to prevent the continuation application from receiving benefit of parent's date).

[5] Claims 1 and 4 present little difficulty. Claim 1 recites a solids content range of "at least 35%," which reads literally on embodiments employing solids contents outside the 25-60% range described in the Swiss application. As in cases involving the enablement requirement of § 112, e. g., *In re Armbruster*, 512 F.2d 676, 185 USPQ 152 (Cust. & Pat.App.1975), we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims. By pointing to the fact that claim 1 reads on embodiments outside the scope of the description, the

PTO has satisfied its burden. Appellants thus have the burden of showing that the upper limit of solids content described, i. e., 60%, is inherent in "at least 35%," as that limitation appears in claim 1. Appellants have adduced no evidence to carry this burden as to claim 1, and they argue only that since the Pfluger patent contains claim 1 supported by Pfluger's disclosure with a stated upper limit of 60%, like appellants' Swiss disclosure, refusal to grant appellants claim 1 amounts to an illegal reexamination of claim 1 in Pfluger. However, as we have often repeated, as recently as *In re Giolito*, 530 F.2d 897, 188 USPQ 645 (Cust. & Pat. App. 1976), it is immaterial in ex parte prosecution whether the same or similar claims have been allowed to others.

[6] Claim 4 contains the additional limitations, relating to the "final product temperature" and the pressure at which the frozen foam is vacuum freeze-dried, of "less than 110°F." and "less than 500 microns." "Final product temperature," it appears, refers to the temperature at which so-called bound water is driven off from the product by heating after the vacuum drying phase has ended. We find no description of final product temperature in appellants' Swiss application. It is not disputed that appellants do not expressly disclose final product temperatures or this secondary drying step. They again appeal, however, to the Pfluger patent disclosure and to an amendment entered in the application on appeal (not objected to as new matter by the examiner) to show that final product temperatures are conventional in the art and need not be expressly disclosed. The amendment is clearly irrelevant since claim 4, an originally filed claim, is its own written description in the appealed application. *In re Gardner*, 475 F.2d 1389, 177 USPQ 296, rehearing denied, 480 F.2d 879, 178 USPQ 149 (Cust. & Pat. App. 1973). The issue is whether the Swiss application describes the claimed final product temperature, not whether the instant application does so. The Pfluger

patent disclosure is also unavailable to appellants. The Swiss application was filed before Pfluger issued, which means that for the purposes of § 112 the Pfluger disclosure is not evidence of what those skilled in the art considered conventional at the time the Swiss application was filed. *In re Glass*, 492 F.2d 1228, 181 USPQ 81 (Cust. & Pat. App. 1974).⁷

[7] Claims 1 and 4, therefore, are not entitled to the benefit of the filing date of appellants' Swiss application.

[8] Claims 2, 37, and 38, which claim a solids content range of "between 35% and 60%," present a different question. They clearly claim a range within the described broad range of 25% to 60% solids; the question is whether, on the facts, the PTO has presented sufficient reason to doubt that the broader described range also describes the somewhat narrower claimed range. We note that there is no evidence, and the PTO does not contend otherwise, that there is in fact any distinction, in terms of the operability of appellants' process or of the achieving of any desired result, between the claimed lower limit of solids content and that disclosed in the Swiss application. We see an important practical distinction between broad generic chemical compound inventions, for example, as in *In re Ruschig*, *supra*, in which each compound within the genus is a separate embodiment of the invention, and inventions like that at bar, in which the range of solids content is but one of several process parameters. What those skilled in the art would expect from using 34% solids content in the concentrated extract prior to foaming instead of 35% is a different matter from what those skilled in the art would expect from the next adjacent homolog of a compound whose properties are disclosed in the specification. We wish to make it clear that we are not creating a rule applicable to all description requirement cases involving

7. That the final product temperature limitation is not material, as appellants argue, does not matter when the limitation is copied. Inmaturity excuses only failure to copy a limitation

of a patent claim. See *Brailsford v. Lavel*, 318 F.2d 942, 56 CCPA 1367, 138 USPQ 28 (1963); 37 CFR 1.265(a).

ranges. Where it is clear, for instance, that the broad described range pertains to a different invention than the narrower (and subsumed) claimed range, then the broader range does not describe the narrower range. *In re Baird*, 348 F.2d 974, 52 CCPA 1747, 146 USPQ 579 (1965); *In re Draeger*, 150 F.2d 572, 32 CCPA 1217, 66 USPQ 247 (1945).

In the context of this invention, in light of the description of the invention as employing solids contents within the range of 25-80% along with specific embodiments of 38% and 50%, we are of the opinion that, as a factual matter, persons skilled in the art would consider processes employing a 85-90% solids content range to be part of appellants' invention and would be led by the Swiss disclosure so to conclude. Cf. *In re Kusching*, *supra*. The PTO has done nothing more than to argue lack of literal support, which is not enough. If lack of literal support alone were enough to support a rejection under § 112, then the statement of *In re Lukach*, *supra*, 442 F.2d at 969, 58 CCPA at 1235, 169 USPQ at 796, that "the invention claimed does not have to be described *in ipsius verbis* in order to satisfy the description requirement of § 112," is empty verbiage. The burden of showing that the claimed invention is not described in the specification rests on the PTO in the first instance, and it is up to the PTO to give reasons why a description *not in ipsius verbis* is insufficient.

[9] We conclude, therefore, that claims 2, 37, and 38 are entitled to the benefit of the filing date of appellants' Swiss application.

Since the Pfluger patent is not available as prior art as of its 1966 date under §§ 102(e) and 103 against claims 2, 37, and 38, the rejection of those claims is reversed. The rejection of claims 1 and 4 is affirmed. Appellants filed no affidavit under Rule 204(c) showing a date of invention for claims 1 and 4 prior to Pfluger's 1966 filing date, *In re Gemasemer*, 319 F.2d 539, 51 CCPA 728, 188 USPQ 229 (1963), and have not antedated Pfluger as to those claims under 35 U.S.C. §§ 119 and 120.

The New Matter Rejection

[10] The issue to be decided here is whether the limitation appearing in claim 6, carried forward into the other claims affected by this rejection, that the frozen foam be ground "to a particle size of at least 0.25 mm" before it is dried, was added to the instant application in violation of 35 U.S.C. § 132. This new matter rejection rests on a finding by the PTO that the application as filed did not describe this limitation. Thus, the converse of what we said in *In re Bowen*, 492 F.2d 859, 864, 181 USPQ 48, 52 (Cust. & Pat. App. 1974), is true in this case, namely, that this new matter rejection is tantamount to a rejection of the claims on the description requirement of 35 U.S.C. § 112, first paragraph. The solicitor agrees with this.

We conclude that the originally filed specification clearly conveys to those of ordinary skill in the art that appellants invented processes in which the frozen foam is ground to a particle size of "at least .025 mm," and not, as the PTO asserts, only processes in which the particle sizes are no larger than 2 mm. See *In re Smythe*, *supra*.

The specification states, *inter alia* (emphasis ours):

At the end of the [cooling] belt the extract is removed as a continuous rigid sheet which may then be broken up into fragments suitable for grinding. These fragments may, for example, be ground to a particle size which is preferably within the range 0.25 to 2.0 mm.

* * * * *

In a modification of the process, the frozen extract may be freeze-dried in the form of plates or lumps which are subsequently ground to the desired particle size.

The examples speak of drying frozen ground particles of sizes between 0.1 and 2 mm. While the specification indicates that the 0.25 to 2.0 mm range is preferred, we think it clearly indicates that, as an alternative embodiment of appellants' invention,

the foam may be dried in lumps or plates of undisclosed size, which are reduced to the obviously smaller preferred particle size by grinding only after being dried. The solicitor argues that the claimed "range" has no upper limit, wherefore it is not disclosed. The clear implication of this disclosed modification is that appellants' specification does describe as their invention processes in which particle size is "at least 0.25 mm," without upper limit, as delineated by the rejected claims. The rejection of claims 6-10, 12-15, 17, and 26 under 35 U.S.C. § 132 is reversed.

The "Non-Interference" Claims—6-25 and 40-43

In the Examiner's Answer, appellants were granted the benefit of the filing date of their Swiss application for claims 18-23, 27-35, and 40-43. The examiner stated: "Claims 6-15 and 26, except for new matter, would otherwise be supported in the Swiss application." Our reversal of the new matter rejection eliminates the basis for the examiner's refusal to give claims 6-15 and 26 the benefit of appellants' Swiss filing date. Appellants' parent and Swiss applications contain the same disclosures concerning particle size as does the application on appeal, and we shall treat all the claims under this heading as entitled to the right of foreign priority claimed by appellants.

Our analysis of these claims will be broken down by the type of claim involved, i. e., process, apparatus, and product, and not as the board addressed them. In each discussion we will apply as prior art under § 102(e) only those portions of the Pfluger patent disclosure that were carried forward from the Pfluger 1963 application (Pfluger 1963) through the two subsequent applications into the patent, as did the board. *In re Lund*, *supra*.

A. Process Claims 6-14 and 16-29

There are four independent process claims: claim 6, from which claims 7-14, 16, and 17 depend; claim 18; claim 19, from which claim 20 depends; and claim 21, from which claims 22-29 depend.

Pfluger 1963 contains the following disclosure, which, in substance, is carried forward into the patent:

This invention is founded on the discovery that an aqueous aromatic liquid containing solids in suspension and solution may be dried without undergoing loss of aromatic volatiles by a process which comprises foaming the aqueous liquid to a substantial overrun while avoiding evaporation of said aqueous liquid, freezing said foam to below its eutectic point while avoiding evaporation of the aqueous liquid, subliming said aqueous liquid from the frozen foam to reduce the moisture of the foam to at least 10-20%, and further drying the foam to a stable moisture content.

In many applications such foaming can be considerably increased by concentrating the solution or suspension to a relatively high solids content prior to incorporation of air or other gas such as nitrogen therein by first whipping and then freezing the foam, preferably by conductive freezing. During the foaming step, it is essential in order to prevent loss of volatiles to avoid any evaporative cooling of the material, i. e., evaporation of water during the foaming step. Also, during the freezing step evaporative cooling should be avoided. Other ways for creating a frozen foam without undergoing evaporative cooling involve the overt introduction to a solution or suspension of dry ice, i. e., solid carbon dioxide in a suitably ground or particulate form, whereby carbon dioxide gas is liberated upon subliming of the "dry ice" to cause foaming of the solution or suspension to occur. Similarly, refrigerated air or nitrogen can be introduced to the solution or suspension to cause freezing thereof incident to foaming the material. The foam preferably has a high overrun whereby the density of the solution or suspension is changed from above 1.0 gm./cc. to between 0.1-0.5 gms/cc.

Example I, the sole disclosed embodiment in which the foam density is given, shows

foaming the extract to a density of 0.22 gm/cc.

Claims 19 and 20 recite a foam density of "between about 0.6 and about 0.8 gm/cc," outside the range disclosed by Pfluger 1963. The examiner's position was that Pfluger's disclosure of 0.5 gm/cc as an upper density limit suggests "about 0.6 gm/cc" as the lower limit in the processes of claims 19 and 20 "in the absence of a critical difference between them." We see no such suggestion. By preferring a high foam overrun, i.e., lower rather than higher foam densities, Pfluger 1963 teaches away from employing higher foam densities than its disclosed upper limit of 0.5 gm/cc. Appellants' "about 0.6 gm/cc" lower limit is sufficiently precise to describe foam densities above 0.5 gm/cc and thus outside the range of foam densities that persons of ordinary skill in the art would have been motivated to use by Pfluger 1963's disclosure of a preference for high overrun foams no denser than 0.5 gm/cc. The examiner's comment about the lack of a showing of a critical difference is based on his failure to appreciate that Pfluger 1963 teaches away from increasing foam density. The rejection of claims 19 and 20 under § 103 is reversed.

[11, 12] Claims 6-14, 16, 17, and 21-29 recite foam density ranges of "between about 0.4 and 0.8 gm/cc" and solids contents in the range of "about 25% to 60%." Claims 6-10, 12-14, 17, and 26 recite particle sizes of "at least 0.25 mm," claims 16 and 27 say "about 0.25 to 2 mm," claims 11 and 28 recite particle sizes "approximately equal to that of roast and ground coffee," and claims 21-25 do not mention particle size. Pfluger 1963's disclosed foam density range of 0.1-0.5 gm/cc covers values within the scope of all the above-listed claims; the solids contents disclosed in Pfluger 1963 Examples I (27%) and V (30%) are within the claimed ranges of 25-60%. Pfluger 1963 clearly teaches a process for making instant coffee comprising the steps of preparing and concentrating aqueous coffee extract, foaming the extract then freezing the foam, and drying the frozen foam, in that order. Pfluger 1963 teaches fragment-

ing the frozen foam into $\frac{1}{4}$ -inch pieces before drying; $\frac{1}{4}$ inch is, of course, "at least 0.25 mm." Of course, the disclosure in the prior art of any value within a claimed range is an anticipation of the claimed range. We appreciate the arguments made in *In re Malagari*, 499 F.2d 1297, 182 USPQ 549 (Cust. & Pat. App. 1974), and the discussion *In re Orfeo*, 440 F.2d 439, 58 CCPA 1123, 169 USPQ 487 (1971), to the effect that ranges which overlap or lie inside ranges disclosed by the prior art may be patentable if the applicant can show criticality in the claimed range by evidence of unexpected results. The rejections here are under § 103, not § 102, which requires us to consider appellants' argument that their invention and Pfluger's disclosure are directed to different purposes and that persons of ordinary skill in the art would not look to Pfluger 1963 for a solution to the problem addressed by appellants. See *In re Orfeo*, *supra*.

Appellants' contentions were thus stated in their main brief:

The Board erred at the threshold in failing to appreciate that neither the Pfluger patent nor the 1963 Pfluger application gives any inkling or hint of the inventive concept underlying the rejected claims. * * * The Pfluger disclosures make no mention of product bulk density and contain no suggestion of altering or regulating that density in any manner. Neither does the reference suggest appellants' step of grinding the foam before freeze drying.

One of ordinary skill in the art reading the 1963 Pfluger disclosure would have no inkling of the problem addressed and solved by appellants; and one looking for ways to meet that problem would have no occasion to consider Pfluger or his expedients.

Without an antecedent basis for it in their application, appellants may not use this rationale to show unobviousness. *In re Davies*, 475 F.2d 667, 177 USPQ 381 (Cust. & Pat. App. 1973). While appellants do disclose what the bulk density of their product

"usually" is, we find no suggestion in appellants' application that their invention is addressed to the regulation of the bulk density of the product, and the claims make no express reference to such regulation. The only references in appellants' disclosure to this alleged problem and its solution which are apparent to us are (emphasis ours):

After freeze-drying, the coffee extract is obtained in the form of a powder the density of which is usually 0.2 to 0.3 gm/cc.

* * * * *

Drying of the concentrated extract should desirably be carried out *under controlled conditions* such that the finished product possesses an appropriate density and colour. * * *

* * * The conditions of freezing, notably belt speed, freezing temperature, thickness of foam layer as well as the density of the foam, are factors which have an important influence on the colour of the finished product and should therefore be carefully controlled.

The inadequacy of this disclosure is evident. There is no mention of regulating the final product density or of controlling solids content. We therefore see no basis for depreciating Pfluger as evidence of the scope and content of the prior art, as well as of the level of ordinary skill in this art, as appellants would have us do. Nor is there any factual basis for concluding that the ranges claimed by appellants are critical in themselves to their alleged inventive contribution.

We find no error in the rejection under § 103 of claims 6-14, 16, and 21-28, which recite no final product density. The only difference between claims 6, 12-14, and 16 and the Pfluger 1963 disclosure upon which appellants rely to show the unobviousness of the subject matter of the claims (and which does not relate to solids content or foam density) is the step of "grinding the frozen foam to a particle size of at least 0.25 mm" prior to freeze-drying.⁸ Pfluger 1963, appellants assert, "fragments" the

frozen foam prior to drying and "grinds" the foam only after it has been dried. As indicated above, the size of the fragments of frozen foam disclosed by Pfluger 1963 is "at least 0.25 mm." We do not think this difference shows the subject matter to be unobvious. Pfluger 1963 implies that the sizes of foam particles before and after drying are comparable; it would have been obvious to reduce the size of the foam particles by suitable mechanical means, whether it be called fragmenting or grinding, to the desired end product size before rather than after drying. Claim 11 differs only in its recitation of final product particle size, which Pfluger 1963 shows is an obvious matter of choice for those of ordinary skill in the art, who know how large ground roasted coffee bean particles are. The commercial motivation for making the particles this size is obvious. Appellants have not argued the patentability separately from claim 6 of claims 9 and 10, which add temperature and foam thickness limitations suggested by Pfluger and De George, as discussed *infra* in considering claims 24 and 25.

Claim 8 likewise recites no final product density, but it requires that the freezing of the foam take place over a period of 7 to 25 minutes, which, appellants' application indicates, produces instant coffee "having a pleasant dark colour." Pfluger 1963 discloses freezing in liquid nitrogen or liquid air, which would be instantaneous, or rapid freezing on a belt, and states further, "The foam may be frozen at a high or a more gradual rate *without any apparent difference* in the utility thereof insofar as freeze drying is concerned" * * *. (Emphasis ours.) Appellants have not shown that only their claimed freezing time produces coffee with a pleasant dark color. Thus, they have not overcome the *prima facie* case of obviousness made out by Pfluger 1963.

In light of appellants' concession in the amendment in which they added claims 27-29 that freeze concentration was known in the art, the rejection of claims 21-23, and

8. Appellants do not deny that the features added in claims 7, 12, 13, and 14 are taught in the

art, and the record shows them to be known in the prior art.

26-28 under Category VI, *supra*, becomes little more than a rejection on Pfluger 1963 alone. With the exception of freeze concentration, which is disclosed by the British patent, every element of claim 21 is disclosed by Pfluger 1963, as indicated *supra*. Appellants advance no arguments for the patentability of claim 21 different from those we have already rejected for claim 6. Claim 22 adds only a recitation of the inert gases used in the foaming step, which were known in the prior art. Claims 26-28 recite the particle sizes of claims 6, 16, and 11, respectively; these particle sizes are not sufficient to show unobviousness for the reasons given *supra*. Claim 23, which was also rejected under Category VI, recites the freezing time of claim 8. It is unpatentable for the same reasons given for claim 8, *supra*.

Claims 24 and 25, to which Pfluger 1963, De George, and the British patent were applied under § 103, call for the temperature and foam limitations already discussed under claims 9 and 10, *supra*. Temperature and foam thicknesses within the claimed ranges are disclosed by Pfluger 1963 in Example VI (freezing foam at -30° F. on a belt and subsequently loading foam onto trays to a 1-inch (approx. 25 mm) depth for vacuum drying). Appellants do not allege that the ranges of claims 24 and 25 are critical.

Claims 17, 18, and 29, on the other hand, recite the bulk density of the final product made by each process in positive terms. The board dismissed these final product density limitations as being merely recitations of the inherent result of observing the foam density and solids content ranges set forth in these claims. Although we found above that appellants' specification as filed does not disclose regulating product density by controlling the foam density and solids content in the process and that claims which failed to recite controlled product density could not rely on this feature to distinguish over the prior art under § 103, these claims do require such regulation or control, by implication through their ex-

press recitation of the density of the final product to be obtained from the processes they delimit. That persons skilled in the art may not know how to ensure the claimed final product densities from the specification is pertinent only to a rejection on the enablement requirement of § 112, first paragraph, which is not before us. The only question here is whether the subject matter of claims 17, 18, and 29, the scope of which is unquestionably clear, is obvious under § 103.

Pfluger 1963 discloses no final product densities and contains no teaching on how to achieve any particular final product density from practicing its process. The inherency of final product density adverted to by the board can be gleaned only from appellants' disclosure, if anywhere, which may not be used against them as prior art absent some admission that matter disclosed in the specification is in the prior art. *In re Kuehl*, 475 F.2d 658, 177 USPQ 250 (Cust. & Pat.App.1973); cf. *In re Nomiya*, 509 F.2d 566, 184 USPQ 697 (Cust. & Pat.App.1973). In the absence of disclosure of final product densities or how to achieve any desired density in the prior art applied by the PTO to claims 17, 18 and 29, we cannot say that the subject matter of these claims would have been obvious to persons of ordinary skill in the art.

The rejection of process claims 6-14, 16, and 21-28 is affirmed; the rejection of claims 17-20, and 29 is reversed.

B. Apparatus Claims 30-35

[13] The preamble of independent claim 30, carried forward into claims 31-35, recites that the apparatus is "for carrying out the process in claim 6." Appellants contend that this preamble gives "life and meaning" to the claims, serving to define the interrelationship of the mechanical elements recited in the body of the claims. This argument appears to be based on *Kropa v. Robbie*, 187 F.2d 156, 38 CCPA 858, 88 USPQ 478 (1951), the classic case in this court on the construction of claim preambles. In *Kropa* the court surveyed prior cases and

ssid, 187 F.2d at 152, 38 CCPA at 861, 38 USPQ at 489-91;

[I]t appears that the preamble has been denied the effect of a limitation where the claim or count was drawn to a structure and the portion of the claim following the preamble was a self-contained description of the structure not depending for completeness upon the introductory clause * * *. In these cases, the claim or count apart from the introductory clause completely defined the subject matter, and the preamble merely stated a purpose or intended use of that subject matter.

While we do not subscribe to the broad proposition that process limitations can never serve to distinguish the subject matter of apparatus claims from the prior art, we fail to see how the general process parameters of claim 6 require an arrangement of the apparatus means recited in claims 30-35 more specific than that set forth in the body of each claim. In no claim is the preamble relied on to provide an antecedent basis for terms in the body. See *In re Higbee*, 527 F.2d 1405, 188 USPQ 488 (Cust. & Pat.App.1976). The context of each invention is clear without reference to claim 6, unlike the situation in *Kropa*, *supra*, in which the preamble "An abrasive article" was the only portion of the claim defining the relationship of the components recited in the body of the claim; the court said, "The term calls forth a distinct relationship between the proportions of grain and resin comprising the article." 187 F.2d at 152, 38 CCPA at 862, 38 USPQ at 481.

Appellants do not argue the patentability of claims 32-35 separately from claim 30 and concede that Carpenter discloses the feature added in claim 31. We find that the teachings of Pfluger and De George (and Carpenter on claim 31) show that the subject matter of claims 30-35 would have been obvious to persons of ordinary skill in the art. These references are to be viewed for what they disclose in their entireties and not merely for their inventive contributions to the art. *In re Oginie*, 517 F.2d 1382, 1287, 186 USPQ 227, 232 (Cust. & Pat.App. 1975).

Pfluger 1963, in a portion carried forward to the patent, discloses the following:

Advantageously, in following the teachings of the present process either in a vacuum freeze drying application or in an atmospheric freeze drying application, the frozen foamy mass may be arranged for either batch or continuous processing in any one of a variety of conventional plant handling applications. Thus, the foamy mass can be readily transferred from one food handling station to another, deposited in trays or continuous belts, superposed on one another or otherwise conventionally located in the vicinity of the freeze drying influences. In the case of a typical freeze drying operation the foams may be frozen and deposited onto trays stacked one above the other on a suitable heat transfer surface in a vacuum chamber. In the case of an atmospheric freeze drying application the foams can be stacked one upon the other upon a foraminous drying member permitting the circulation of the drying medium, e. g. dry air, helium or nitrogen. Throughout all of such freeze drying applications it is imperative that the temperature of the foamy mass be maintained below the eutectic point of the material while drying to assure that the foam stays in a substantially solid or frozen state as distinguished from a melted or semi-liquid state, dehydration of the mass being achieved by a process of sublimation as distinguished from one of evaporation. Such conditions should be followed at least until the moisture content of the foamy mass has been substantially reduced to a point where it has lost at least a majority of its moisture and preferably is superficially dry to the touch, i. e. in the neighborhood of 10-20% moisture by weight.

Example VI of Pfluger 1963, which is carried forward as Example III of the Pfluger patent, shows heat controlling the vacuum chamber to assure a product temperature below -10° F. (De George teaches that the melting point of a 28% solids content extract is about 27° F., whereas the eutectic temperature is constant regardless of con-

centration at about -13.5°F.) De George discloses the use of endless belts, low speeds, and refrigerating means, and appellants, while arguing that De George treats the handling of solid slabs of frozen extract on refrigeration belts and not frozen foamed extracts, do not and cannot deny that De George discloses apparatus that persons of ordinary skill in the art would have deemed suitable for handling foams in the manner shown by Pfluger. Appellants also contend that neither reference discloses the "spreading device" recited in the claims, Pfluger 1963 showing only the application of $\frac{1}{8}$ " diameter ribbons of foam through a nozzle to stationary freeze drying trays. The reference in the portion of Pfluger 1963 quoted *supra* to the deposition of the foam on the belts is ample suggestion, in our opinion, that some means must be employed to apply the foamy mass to the continuous belts. The term "spreading device" is not defined in any special way by appellants and is broad enough to be the means for applying the foam to the belt suggested by Pfluger. The rejection of claims 30-35 is affirmed.

C. Product Claims 15 and 40-43

[14] These claims are cast in product-by-process form. Although appellants argue, successfully we have found, that the Pfluger 1963 disclosure does not suggest the control of bulk density afforded by appellants' process, the patentability of the products defined by the claims, rather than the processes for making them, is what we must gauge in light of the prior art. See *In re Bridgeford*, 337 F.2d 679, 63 CCPA 1182, 149 USPQ 55 (1966). Each of these claims defines a freeze-dried instant coffee product made by processes which, appellants have contended with respect to their process claims, produce, by virtue of the foam density and solids content ranges taught by

⁹ Appellants argue in their reply brief that claims 40-43 "were never the subject of an accurate or proper rejection," because the examiner and the board incorrectly grouped them with other claims. As we have indicated, the rejection of claims 40-43 on Pfluger under § 103 was "proper"; appellants do not contend that they could not understand the basis for the

appellants, products having a bulk density comparable to spray-dried instant coffee, i. e., 0.2-0.3 gm/cc as indicated in appellants' specification. The solids content and foam density ranges disclosed by Pfluger 1963 overlap those of appellants, and, it appears, the Pfluger process using solids contents and foam densities overlapping those of appellants will produce instant coffee which is indistinguishable from appellants' products. There is no evidence showing that Pfluger's product prepared, for example, using an extract of 30% solids content foamed to a density of 0.5 gm/cc differs from appellants' claimed products in any way, certainly not in any obvious way. See *In re Avery*, 518 F.2d 1228, 1233-34, 186 USPQ 161, 165-66 (Cust. & Pat.App.1978). That some of the products covered by appellants' claims may not be disclosed or suggested by Pfluger 1963 is not relevant to patentability, since the claims embrace other subject matter completely disclosed by Pfluger 1963, complete disclosure in the prior art being the epitome of obviousness. *In re Pearson*, 494 F.2d 1299, 181 USPQ 641 (Cust. & Pat.App.1974). The rejection of these product claims under § 103 on Pfluger⁹ is affirmed.

Conclusion

The appeal is dismissed as to withdrawn claims 3, 5, 36, and 39. The decision of the board is affirmed as to claims 1, 4, 6-16, 21-28, 30-35 and 40-43, and is reversed as to claims 2, 17-20, 29, 37, and 38.

MODIFIED

APPENDIX

2. The process of claim 1 wherein the extract is concentrated to between 35% and 60% soluble solids prior to the foaming step.

rejection because of failure of the PTO to give clear reasons for its action under 35 U.S.C. § 132, and we find the explanations given by the examiner and board with respect to claims 40-43 to have been legally ample under § 132. Cf. *In re Gustafson*, 331 F.2d 965, 51 CCPA 1328, 141 USPQ 585 (1964).

APPENDIX—Continued

3. The process of claim 2 wherein the concentrated extract is foamed to an overrun density of between 0.1 to 0.7 gm/cc.
4. The process of claim 2 wherein the frozen foam is vacuum freeze-dried at a pressure of less than 500 microns and a final product temperature of less than 110°F.
5. The process of claim 3 wherein the frozen foam is vacuum freeze-dried at a pressure of less than 500 microns and a final product temperature of less than 110°C.
7. A process according to claim 6 in which said inert gas is at least one of the following gases, namely carbon dioxide, nitrous oxide and nitrogen.
8. A process according to claim 6 in which the foam is frozen during 7 to 25 minutes.
9. A process according to claim 6 in which the foam is frozen on a moving belt which is cooled to a temperature between -12 and -70°C.
10. A process according to claim 6 wherein the foam is spread on the belt at a layer thickness of 10 to 40 mm.
11. A process according to claim 6 in which the frozen foam is ground, before freeze-drying, to a particle size approximately equal to that of roast and ground coffee.
12. A process according to claim 6 in which an aromatic condensate obtained by stripping roast and ground coffee is added to said concentrated extract before it is transformed into a foam.
13. A process according to claim 6 in which, after freeze-drying, the powdered coffee extract is aromatised by incorporation therein of 0.1 to 0.5% by weight of an aromatic condensate obtained by stripping of roast and ground coffee.
14. A process according to claim 13 in which said condensate is incorporated in said powdered extract in admixture with an oily carrier.
15. The coffee extract obtained by the process defined in claim 6.
16. Process according to claim 6 in which the frozen foam is ground to a particle size of about 0.25 to 2.0 mm.
17. Process according to claim 6 in which the freeze dried extract has a density of about 0.2 to 0.3 gm/cc.
18. Process for preparing a soluble coffee extract, which comprises adding inert gas to a concentrated aqueous extract of roast coffee having a solids content of about 25% to about 60% to provide a foam, freezing the foam to a solid mass, reducing the frozen foam to particles having a size of about 0.25 to 2.0 mm and freeze drying the frozen particles, the amount of inert gas added to the aqueous extract being sufficient to provide a freeze dried extract having a density between about 0.2 and 0.8 gm/cc.
19. Process for preparing a powdered coffee extract which comprises adding sufficient inert gas to a concentrated aqueous extract of roast coffee to provide a foam having a density between about 0.6 and about 0.8 gm/cc, freezing the foamed extract to a solid mass, grinding the frozen foam to an average particle size of 0.1 to 0.5 mm, freeze drying the ground particles to provide a finely powdered coffee extract, and agglomerating the finely powdered coffee extract.
20. Process according to claim 19, in which the powdered extract is agglomerated to provide an agglomerate having a density of about 0.2 to 0.3 gm/cc.
21. Process for preparing a powdered coffee extract which comprises increasing the soluble coffee solids content of an aqueous extract of roast ground coffee to about 25%–60% by freeze concentration, separating the concentrated extract from ice crystals, adding an inert gas to the concentrated aqueous extract to provide a foam having a density between about 0.4 and 0.8 gm/cc, freezing the foam to a solid mass and freeze drying the frozen foam.
22. Process according to claim 21 in which the inert gas is selected from the group consisting of carbon dioxide, nitrous oxide and nitrogen.

APPENDIX—Continued

23. Process according to claim 21 in which the foam is frozen during 7 to 25 minutes.

24. Process according to claim 21 in which the foam is frozen on a moving belt which is cooled to a temperature between -12 and -70°C.

25. Process according to claim 24 wherein the foam is spread on the belt at a layer thickness of 10 to 40 mm.

26. Process according to claim 21 in which the frozen foam is ground before freeze drying to a particle size of at least 0.25 mm.

27. Process according to claim 26 in which the frozen foam is ground to a particle size of about 0.25 to 2mm.

28. Process according to claim 21 in which the frozen foam is ground before freeze drying to a particle size approximately equal to that of roast and ground coffee.

29. Process according to claim 21 in which the freeze dried extract has a density of about 0.2-0.3 gm/cc.

30. An apparatus according to claim 30 in which the means for cooling the belt includes a plurality of sprinklers disposed to spray the refrigerant onto the underside of the belt.

31. An apparatus according to claim 30 in which the belt comprises two sections each provided with separate cooling means, the first of said sections being cooled to a temperature of -12 to -20°C and the second section to -40 to -70°C.

32. An apparatus according to claim 30 also comprising means for fragmenting and milling the frozen foam.

33. An apparatus according to claim 30 in which the length of said belt is 15 to 25 metres and the driving means is adapted to move said belt at a linear speed of about 0.5 to 1.5 m/min.

34. An apparatus according to claim 30 in which said chamber is adapted to be maintained at a temperature of -25 to -45°C.

35. The process of claim 2 wherein the concentrated extract is foamed to an overrun density of between about 0.1 to 0.8 gm/cc.

36. The process of claim 2 wherein the concentrated extract is foamed to an overrun density of between 0.4 to 0.8 gm/cc.

37. The process of claim 2 wherein the concentrated [806] extract is foamed to an overrun density of between 0.4 to 0.8 gm/cc.

38. The process of claim 2 wherein the frozen foam is vacuum freeze-dried at a pressure of about 150 to 175 microns.

39. The process of claim 3 wherein the frozen foam is vacuum freeze-dried at a pressure of about 150 to 175 microns.

40. A coffee powder according to claim 40 wherein the extract before freeze drying contains about 25% to 60% by weight of soluble coffee solids.

41. A dry coffee powder having a density of about 0.2 to 0.3 gm/cc and comprising a freeze dried particulated foamed extract of roast and ground coffee, said extract containing before freeze drying up to about 60% by weight of soluble coffee solids.

42. A coffee powder according to claim 42 containing about 0.1% to 0.5% by weight of aromatic condensate obtained by stripping roast and ground coffee.

BALDWIN, Judge (concurring in part and dissenting in part).

I agree with Judge Miller's treatment of claims 17-20 and 29. Otherwise, I join the majority opinion.

MILLER, Judge (dissenting in part and concurring in part).

I dissent on claim 1. The error of the majority in affirming the rejection stems from a misstatement of the issue. It is not necessary when antedating a reference under 35 U.S.C. § 102(a) or (e) to establish a prior reduction to practice, constructive or actual, of all the subject matter falling within the claims. It is necessary only to establish a reduction to practice of sufficient subject matter to render the claimed invention obvious to one of ordinary skill in the art. *In re Spiller*, 500 F.2d 1170, 182 USPQ 614 (Cust. & Pat.App.1974). The

majority errs, therefore, in seeking a description in appellants' parent and foreign priority applications to support the entire claimed subject matter as though these were the applications in which the claims appear. See *In re Ziegler*, 347 F.2d 642, 52 CCPA 1473, 146 USPQ 76 (1965). Appellants have clearly shown possession of enough of the invention to antedate Pfluger 1966 by establishing a prior constructive reduction to practice in their parent and foreign applications of specific embodiments disclosing concentrating to 50% and 38% total solids and by a broader disclosure of "25 to 60%."

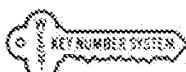
Although the rejection of claim 1 arises in the context of an attempt to initiate an interference, the rejection is clearly under 35 U.S.C. § 102(a) or (e) and not under Rule 204(c), 37 CFR 1.204(c). Even if the rejection were under that rule, the substance of the rule's requirement for evidence sufficient to establish a *prima facie* case for a judgment of priority against Pfluger 1966 would be satisfied by the prior constructive reduction to practice of embodiments within claim 1 in appellants' parent and foreign applications. *Hunt v. Treppschuh*, 523 F.2d 1386, 187 USPQ 426 (Cust. & Pat.App.1975); *Fontijn v. Okamoto*, 518 F.2d 610, 186 USPQ 97 (Cust. & Pat.App.1975).

The majority cites *In re Gemassmer*, 319 F.2d 539, 51 CCPA 726, 188 USPQ 229 (1963), to support its decision on claim 1. It suffices to note that Gemassmer was decided more than a decade before *In re Spiller*, *Hunt v. Treppschuh*, and *Fontijn v. Okamoto*, *supra*.

I concur in the decision on claim 4 since appellants' parent and foreign applications are silent regarding final product temperature and a secondary heating step and, therefore, fail even as a constructive reduction to practice of the invention of claim 4.

I concur also in the decision on claims 19 and 20, but I do not find it necessary to hold, as the majority implicitly does, that "about 0.6" gm/cc excludes 0.5 gm/cc disclosed in the reference as the upper limit of merely a preferred range. Moreover, it is obvious from the reference that the process would work at a higher density than 0.5, although inferior results might be expected. My concurrence rests on the requirement of claims 19 and 20 of a specific sequence of steps not suggested by the prior art, namely: providing a high density of about 0.6 to about 0.8 gm/cc, grinding to a fine particle size prior to freeze drying, freeze drying, and finally agglomerating the fine particles into larger particles. This achieves a "highly coloured product of regular particle size." There is no suggestion in the prior art of deliberately grinding to a fine size and then agglomerating to a larger size.

I dissent on claims 17, 18, and 29, because there is at least a *prima facie* relationship between product and foam densities. The board noted this by stating that "the freeze dried density of the coffee would be inherent in view of the same range of foam overrun density disclosed by Pfluger." Since the foam densities and other conditions disclosed by Pfluger for the process claimed are approximately the same, appellants should be required either to show that the reference does not achieve the same product densities or to establish criticality. Since they have not done so, I would affirm the rejection of claims 17, 18, and 29.



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2163 Guidelines for the Examination of Patent Applications Under the 35 U.S.C. - 2100 Patentability

2163 Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, para. 1, "Written Description" Requirement [R-5]

The following Guidelines establish the policies and procedures to be followed by Office personnel in the evaluation of any patent application for compliance with the written description requirement of 35 U.S.C. 112. These Guidelines are based on the Office's current understanding of the law and are believed to be fully consistent with binding precedent of the U.S. Supreme Court, as well as the U.S. Court of Appeals for the Federal Circuit and its predecessor courts.

The Guidelines do not constitute substantive rulemaking and hence do not have the force and effect of law. They are designed to assist Office personnel in analyzing claimed subject matter for compliance with substantive law. Rejections will be based upon the substantive law, and it is these rejections which are appealable. Consequently, any perceived failure by Office personnel to follow these Guidelines is neither appealable nor petitionable.

These Guidelines are intended to form part of the normal examination process. Thus, where Office personnel establish a *prima facie* case of lack of written description for a claim, a thorough review of the prior art and examination on the merits for compliance with the other statutory requirements, including those of 35 U.S.C. 101, 102, 103, and 112, is to be conducted prior to completing an Office action which includes a rejection for lack of written description.

I. GENERAL PRINCIPLES GOVERNING COMPLIANCE WITH THE "WRITTEN DESCRIPTION" REQUIREMENT FOR APPLICATIONS

The first paragraph of 35 U.S.C. 112 requires that the "specification shall contain a written description of the invention * * *." This requirement is separate and distinct from the enablement requirement. See, e.g., *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1560, 19 USPQ2d 1111, 1114 (Fed. Cir. 1991). See also *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 920-23, 69 USPQ2d 1886, 1890-93 (Fed. Cir. 2004) (discussing history and purpose of the written description requirement); *In re Curtis*, 354 F.3d 1347, 1357, 69 USPQ2d 1274, 1282 (Fed. Cir. 2004) ("conclusive evidence of a claim's

enablement is not equally conclusive of that claim's satisfactory written description"). The written description requirement has several policy objectives. "[T]he 'essential goal' of the description of the invention requirement is to clearly convey the information that an applicant has invented the subject matter which is claimed." *In re Barker*, 559 F.2d 588, 592 n.4, 194 USPQ 470, 473 n.4 (CCPA 1977). Another objective is to put the public in possession of what the applicant claims as the invention. See *Regents of the University of California v. Eli Lilly*, 119 F.3d 1559, 1566, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997), *cert. denied*, 523 U.S. 1089 (1998). *>"The 'written description' requirement implements the principle that a patent must describe the technology that is sought to be patented; the requirement serves both to satisfy the inventor's obligation to disclose the technologic knowledge upon which the patent is based, and to demonstrate that the patentee was in possession of the invention that is claimed." *Capon v. Eshhar*, 418 F.3d 1349, 1357, 76 USPQ2d 1078, 1084 (Fed. Cir. 2005). Further, the< written description requirement ** promotes the progress of the useful arts by ensuring that patentees adequately describe their inventions in their patent specifications in exchange for the right to exclude others from practicing the invention for the duration of the patent's term.

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. See, e.g., *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003); *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116. However, a showing of possession alone does not cure the lack of a written description. *Enzo Biochem, Inc. v. Gen-Probe, Inc.*, 323 F.3d 956, 969-70, 63 USPQ2d 1609, 1617 (Fed. Cir. 2002). Much of the written description case law addresses whether the specification as originally filed supports claims not originally in the application. The issue raised in the cases is most often phrased as whether the original application provides "adequate support" for the claims at issue or whether the material added to the specification incorporates "new matter" in violation of 35 U.S.C. 132. The "written description" question similarly arises in the interference context, where the issue is whether the specification of one party to the interference can support the newly added claims corresponding to the count at issue, i.e., whether that party can "make the claim" corresponding to the interference count. See, e.g., *Martin v. Mayer*, 823 F.2d 500, 503, 3 USPQ2d 1333, 1335 (Fed. Cir. 1987). In addition, early opinions suggest the Patent and Trademark Office was unwilling to find written descriptive support when the only description was found in the claims; however, this viewpoint was rejected. See *In re Koller*, 613 F.2d 819, 204 USPQ 702 (CCPA 1980) (original claims constitute their own description); accord *In re Gardner*, 475 F.2d 1389, 177 USPQ 396 (CCPA 1973); accord *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976). It is now well accepted that a satisfactory description may be in the claims or any other portion of the originally filed specification. These early opinions did not address the quality or specificity of particularity that was required in the description, i.e., how much description is enough.

An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997). Possession may be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show

that the applicant was in possession of the claimed invention. See, e.g., *Pfaff v. Wells Elecs., Inc.*, 525 U.S. 55, 68, 119 S.Ct. 304, 312, 48 USPQ2d 1641, 1647 (1998); *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406; *Amgen, Inc. v. Chugai Pharmaceutical*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) (one must define a compound by "whatever characteristics sufficiently distinguish it"). "Compliance with the written description requirement is essentially a fact-based inquiry that will 'necessarily vary depending on the nature of the invention claimed.'" *Enzo Biochem*, 323 F.3d at 963, 63 USPQ2d at 1613. An application specification may show actual reduction to practice by describing testing of the claimed invention or, in the case of biological materials, by specifically describing a deposit made in accordance with 37 CFR 1.801 *et seq.* See *Enzo Biochem*, 323 F.3d at 965, 63 USPQ2d at 1614 ("reference in the specification to a deposit may also satisfy the written description requirement with respect to a claimed material"); see also Deposit of Biological Materials for Patent Purposes, Final Rule, 54 FR 34,864 (August 22, 1989) ("The requirement for a specific identification is consistent with the description requirement of the first paragraph of 35 U.S.C. 112, and to provide an antecedent basis for the biological material which either has been or will be deposited before the patent is granted." *Id.* at 34,876. "The description must be sufficient to permit verification that the deposited biological material is in fact that disclosed. Once the patent issues, the description must be sufficient to aid in the resolution of questions of infringement." *Id.* at 34,880.). Such a deposit is not a substitute for a written description of the claimed invention. The written description of the deposited material needs to be as complete as possible because the examination for patentability proceeds solely on the basis of the written description. See, e.g., *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985). See also 54 FR at 34,880 ("As a general rule, the more information that is provided about a particular deposited biological material, the better the examiner will be able to compare the identity and characteristics of the deposited biological material with the prior art.").

A question as to whether a specification provides an adequate written description may arise in the context of an original claim which is not described sufficiently (see, e.g., *>LizardTech, Inc. v. Earth Resource Mapping, Inc.*, 424 F.3d 1336, 1345, 76 USPQ2d 1724, 1733 (Fed. Cir. 2005); *< Enzo Biochem*, 323 F.3d at 968, 63 USPQ2d at 1616 (Fed. Cir. 2002); *Eli Lilly*, 119 F.3d 1559, 43 USPQ2d 1398), a new or amended claim wherein a claim limitation has been added or removed, or a claim to entitlement of an earlier priority date or effective filing date under 35 U.S.C. 119, 120, or 365(c). Most typically, the issue will arise in the context of determining whether new or amended claims are supported by the description of the invention in the application as filed (see, e.g., *In re Wright*, 866 F.2d 422, 9 USPQ2d 1649 (Fed. Cir. 1989)), whether a claimed invention is entitled to the benefit of an earlier priority date or effective filing date under 35 U.S.C. 119, 120, or 365(c) (see, e.g., *New Railhead Mfg. L.L.C. v. Vermeer Mfg. Co.*, 298 F.3d 1290, 63 USPQ2d 1843 (Fed. Cir. 2002); *Tronzo v. Biomet, Inc.*, 156 F.3d 1154, 47 USPQ2d 1829 (Fed. Cir. 1998); *Fiers v. Revel*, 984 F.2d 1164, 25 USPQ2d 1601 (Fed. Cir. 1993); *In re Ziegler*, 992 F.2d 1197, 1200, 26 USPQ2d 1600, 1603 (Fed. Cir. 1993)), or whether a specification provides support for a claim corresponding to a count in an interference (see, e.g., *Fields v. Conover*, 443 F.2d 1386, 170 USPQ 276 (CCPA 1971)). Compliance with the written description requirement is a question of fact which must be resolved on a case-by-case basis. *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116 (Fed. Cir. 1991).

A. Original Claims

There is a strong presumption that an adequate written description of the claimed invention is present when the application is filed. *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976) ("we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims"). However, as discussed in paragraph I., *supra*, the issue of a lack of adequate written description may arise even for an original claim when an aspect of the claimed invention has not been described with sufficient particularity such that one skilled in the art would recognize that the applicant had possession of the claimed invention. The claimed invention as a whole may not be adequately described if the claims require an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art. For example, consider the claim "A gene comprising SEQ ID NO:1." A determination of what the claim as a whole covers may result in a conclusion that specific structures such as a promoter, a coding region, or other elements are included. Although all genes encompassed by this claim share the characteristic of comprising SEQ ID NO:1, there may be insufficient description of those specific structures (e.g., promoters, enhancers, coding regions, and other regulatory elements) which are also included.

The claimed invention as a whole may not be adequately described where an invention is described solely in terms of a method of its making coupled with its function and there is no described or art-recognized correlation or relationship between the structure of the invention and its function. A biomolecule sequence described only by a functional characteristic, without any known or disclosed correlation between that function and the structure of the sequence, normally is not a sufficient identifying characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence. For example, even though a genetic code table would correlate a known amino acid sequence with a genus of coding nucleic acids, the same table cannot predict the native, naturally occurring nucleic acid sequence of a naturally occurring mRNA or its corresponding cDNA. Cf. *In re Bell*, 991 F.2d 781, 26 USPQ2d 1529 (Fed. Cir. 1993), and *In re Deuel*, 51 F.3d 1552, 34 USPQ2d 1210 (Fed. Cir. 1995) (holding that a process could not render the product of that process obvious under 35 U.S.C. 103). The Federal Circuit has pointed out that under United States law, a description that does not render a claimed invention obvious cannot sufficiently describe the invention for the purposes of the written description requirement of 35 U.S.C. 112. *Eli Lilly*, 119 F.3d at 1567, 43 USPQ2d at 1405. Compare *Fonar Corp. v. General Electric Co.*, 107 F.3d 1543, 1549, 41 USPQ2d 1801, 1805 (Fed. Cir. 1997) ("As a general rule, where software constitutes part of a best mode of carrying out an invention, description of such a best mode is satisfied by a disclosure of the functions of the software. This is because, normally, writing code for such software is within the skill of the art, not requiring undue experimentation, once its functions have been disclosed. * * * Thus, flow charts or source code listings are not a requirement for adequately disclosing the functions of software.").

A lack of adequate written description issue also arises if the knowledge and level of skill in the art would not permit one skilled in the art to immediately envisage the product claimed from the disclosed process. See, e.g., *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1571, 39 USPQ2d 1895, 1905 (Fed. Cir. 1996) (a "laundry list" disclosure of every possible moiety does not constitute a written description of every species in a genus because it would not "reasonably lead" those skilled in the art to any particular species); *In re Ruschig*, 379 F.2d 990, 995, 154 USPQ 118, 123 (CCPA 1967) ("If n-propylamine had been used in making the compound instead of n-butylamine, the compound of claim

13 would have resulted. Appellants submit to us, as they did to the board, an imaginary specific example patterned on specific example 6 by which the above butyl compound is made so that we can see what a simple change would have resulted in a specific supporting disclosure being present in the present specification. The trouble is that there is no such disclosure, easy though it is to imagine it.") (emphasis in original); *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1328, 56 USPQ2d 1481, 1487 (Fed. Cir. 2000) ("the specification does not clearly disclose to the skilled artisan that the inventors ... considered the ratio... to be part of their invention There is therefore no force to Purdue's argument that the written description requirement was satisfied because the disclosure revealed a broad invention from which the [later-filed] claims carved out a patentable portion").

B. New or Amended Claims

The proscription against the introduction of new matter in a patent application (35 U.S.C. 132 and 251) serves to prevent an applicant from adding information that goes beyond the subject matter originally filed. See *In re Rasmussen*, 650 F.2d 1212, 1214, 211 USPQ 323, 326 (CCPA 1981). See MPEP § 2163.06 through § 2163.07 for a more detailed discussion of the written description requirement and its relationship to new matter. The claims as filed in the original specification are part of the disclosure and, therefore, if an application as originally filed contains a claim disclosing material not found in the remainder of the specification, the applicant may amend the specification to include the claimed subject matter. *In re Benno*, 768 F.2d 1340, 226 USPQ 683 (Fed. Cir. 1985). Thus, the written description requirement prevents an applicant from claiming subject matter that was not adequately described in the specification as filed. New or amended claims which introduce elements or limitations which are not supported by the as-filed disclosure violate the written description requirement. See, e.g., *In re Lukach*, 442 F.2d 967, 169 USPQ 795 (CCPA 1971) (subgenus range was not supported by generic disclosure and specific example within the subgenus range); *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) (a subgenus is not necessarily described by a genus encompassing it and a species upon which it reads).

While there is no *in haec verba* requirement, newly added claim limitations must be supported in the specification through express, implicit, or inherent disclosure. An amendment to correct an obvious error does not constitute new matter where one skilled in the art would not only recognize the existence of the error in the specification, but also recognize the appropriate correction. *In re Oda*, 443 F.2d 1200, 170 USPQ 268 (CCPA 1971). With respect to the correction of sequencing errors in applications disclosing nucleic acid and/or amino acid sequences, it is well known that sequencing errors are a common problem in molecular biology. See, e.g., Peter Richterich, Estimation of Errors in 'Raw' DNA Sequences: A Validation Study, 8 *Genome Research* 251-59 (1998). If an application as filed includes sequence information and references a deposit of the sequenced material made in accordance with the requirements of 37 CFR 1.801 *et seq.*, amendment may be permissible. Deposits made after the application filing date cannot be relied upon to support additions to or correction of information in the application as filed. Corrections of minor errors in the sequence may be possible based on the argument that one of skill in the art would have resequenced the deposited material and would have immediately recognized the minor error. Deposits made after the filing date can only be relied upon to provide support for the correction of sequence information if applicant submits a statement in compliance with 37 CFR 1.804 stating that the biological

material which is deposited is a biological material specifically defined in the application as filed.

Under certain circumstances, omission of a limitation can raise an issue regarding whether the inventor had possession of a broader, more generic invention. See, e.g., *PIN/NIP, Inc. v. Platte Chem. Co.*, 304 F.3d 1235, 1248, 64 USPQ2d 1344, 1353 (Fed. Cir. 2002) (Claim for a method of inhibiting sprout growth on tubers by treating them with spaced, sequential application of two chemicals was held invalid for lack of adequate written description where the specification indicated that invention was a method of applying a "composition," or mixture, of the two chemicals.); *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d 1473, 45 USPQ2d 1498 (Fed. Cir. 1998) (claims to a sectional sofa comprising, inter alia, a console and a control means were held invalid for failing to satisfy the written description requirement where the claims were broadened by removing the location of the control means); *Johnson Worldwide Associates v. Zebco Corp.*, 175 F.3d 985, 993, 50 USPQ2d 1607, 1613 (Fed. Cir. 1999) (In *Gentry Gallery*, the "court's determination that the patent disclosure did not support a broad meaning for the disputed claim terms was premised on clear statements in the written description that described the location of a claim element--the 'control means' --as 'the only possible location' and that variations were 'outside the stated purpose of the invention.' *Gentry Gallery*, 134 F.3d at 1479, 45 USPQ2d at 1503. *Gentry Gallery*, then, considers the situation where the patent's disclosure makes crystal clear that a particular (i.e., narrow) understanding of a claim term is an 'essential element of [the inventor's] invention.'"); *Tronzo v. Biomet*, 156 F.3d at 1158-59, 47 USPQ2d at 1833 (Fed. Cir. 1998) (claims to generic cup shape were not entitled to filing date of parent application which disclosed "conical cup" in view of the disclosure of the parent application stating the advantages and importance of the conical shape.). A claim that omits an element which applicant describes as an essential or critical feature of the invention originally disclosed does not comply with the written description requirement. See *Gentry Gallery*, 134 F.3d at 1480, 45 USPQ2d at 1503; *In re Sus*, 306 F.2d 494, 504, 134 USPQ 301, 309 (CCPA 1962) ("[O]ne skilled in this art would not be taught by the written description of the invention in the specification that any 'aryl or substituted aryl radical' would be suitable for the purposes of the invention but rather that only certain aryl radicals and certain specifically substituted aryl radicals [i.e., aryl azides] would be suitable for such purposes.") (emphasis in original). A claim which omits matter disclosed to be essential to the invention as described in the specification or in other statements of record may also be subject to rejection under 35 U.S.C. 112, para. 1, as not enabling, or under 35 U.S.C. 112, para. 2. See *In re Mayhew*, 527 F.2d 1229, 188 USPQ 356 (CCPA 1976); *In re Venezia*, 530 F.2d 956, 189 USPQ 149 (CCPA 1976); and *In re Collier*, 397 F.2d 1003, 158 USPQ 266 (CCPA 1968). See also MPEP § 2172.01.

The fundamental factual inquiry is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the invention as now claimed. See, e.g., *Vas-Cath, Inc.*, 935 F.2d at 1563-64, 19 USPQ2d at 1117.

II. METHODOLOGY FOR DETERMINING ADEQUACY OF WRITTEN DESCRIPTION

A. Read and Analyze the Specification for Compliance with 35 U.S.C. 112, para. 1

Office personnel should adhere to the following procedures when reviewing patent

applications for compliance with the written description requirement of 35 U.S.C. 112, para. 1. The examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed, *Wertheim*, 541 F.2d at 262, 191 USPQ at 96; however, with respect to newly added or amended claims, applicant should show support in the original disclosure for the new or amended claims. See MPEP § 714.02 and § 2163.06 ("Applicant should * * * specifically point out the support for any amendments made to the disclosure."); and MPEP § 2163.04 ("If applicant amends the claims and points out where and/or how the originally filed disclosure supports the amendment(s), and the examiner finds that the disclosure does not reasonably convey that the inventor had possession of the subject matter of the amendment at the time of the filing of the application, the examiner has the initial burden of presenting evidence or reasoning to explain why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims."). Consequently, rejection of an original claim for lack of written description should be rare. The inquiry into whether the description requirement is met is a question of fact that must be determined on a case-by-case basis. See *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) ("Precisely how close [to the claimed invention] the description must come to comply with Sec. 112 must be left to case-by-case development."); *In re Wertheim*, 541 F.2d at 262, 191 USPQ at 96 (inquiry is primarily factual and depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure).

1. For Each Claim, Determine What the Claim as a Whole Covers

Claim construction is an essential part of the examination process. Each claim must be separately analyzed and given its broadest reasonable interpretation in light of and consistent with the written description. See, e.g., *In re Morris*, 127 F.3d 1048, 1053-54, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997). The entire claim must be considered, including the preamble language and the transitional phrase. "Preamble language" is that language in a claim appearing before the transitional phase, e.g., before "comprising," "consisting essentially of," or "consisting of." The transitional term "comprising" (and other comparable terms, e.g., "containing," and "including") is "open-ended" -it covers the expressly recited subject matter, alone or in combination with unrecited subject matter. See, e.g., *Genentech, Inc. v. Chiron Corp.*, 112 F.3d 495, 501, 42 USPQ2d 1608, 1613 (Fed. Cir. 1997) ("'Comprising' is a term of art used in claim language which means that the named elements are essential, but other elements may be added and still form a construct within the scope of the claim."); *Ex parte Davis*, 80 USPQ 448, 450 (Bd. App. 1948) ("comprising" leaves the "claim open for the inclusion of unspecified ingredients even in major amounts"). See also MPEP § 2111.03. "By using the term 'consisting essentially of,' the drafter signals that the invention necessarily includes the listed ingredients and is open to unlisted ingredients that do not materially affect the basic and novel properties of the invention. A 'consisting essentially of' claim occupies a middle ground between closed claims that are written in a 'consisting of' format and fully open claims that are drafted in a 'comprising' format." *PPG Industries v. Guardian Industries*, 156 F.3d 1351, 1354, 48 USPQ2d 1351, 1353-54 (Fed. Cir. 1998). For the purposes of searching for and applying prior art under 35 U.S.C. 102 and 103, absent a clear indication in the specification or claims of what the basic and novel characteristics

actually are, "consisting essentially of" will be construed as equivalent to "comprising." See, e.g., *PPG*, 156 F.3d at 1355, 48 USPQ2d at 1355 ("PPG could have defined the scope of the phrase 'consisting essentially of' for purposes of its patent by making clear in its specification what it regarded as constituting a material change in the basic and novel characteristics of the invention."). See also *AK Steel Corp. v. Sollac*, 344 F.3d 1234, 1239-1240, 68 USPQ2d 1280, 1283-84 (Fed. Cir. 2003); *In re Janakirama-Rao*, 317 F.2d 951, 954, 137 USPQ 893, 895-96 (CCPA 1963). If an applicant contends that additional steps or materials in the prior art are excluded by the recitation of "consisting essentially of," applicant has the burden of showing that the introduction of additional steps or components would materially change the characteristics of applicant's invention. *In re De Lajarte*, 337 F.2d 870, 143 USPQ 256 (CCPA 1964). See also MPEP § 2111.03. The claim as a whole, including all limitations found in the preamble (see *Pac-Tec Inc. v. Amerace Corp.*, 903 F.2d 796, 801, 14 USPQ2d 1871, 1876 (Fed. Cir. 1990) (determining that preamble language that constitutes a structural limitation is actually part of the claimed invention)), the transitional phrase, and the body of the claim, must be sufficiently supported to satisfy the written description requirement. An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations. *Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1966.

The examiner should evaluate each claim to determine if sufficient structures, acts, or functions are recited to make clear the scope and meaning of the claim, including the weight to be given the preamble. See, e.g., *Bell Communications Research, Inc. v. Vitalink Communications Corp.*, 55 F.3d 615, 620, 34 USPQ2d 1816, 1820 (Fed. Cir. 1995) ("[A] claim preamble has the import that the claim as a whole suggests for it."); *Corning Glass Works v. Sumitomo Elec. U.S.A., Inc.*, 868 F.2d 1251, 1257, 9 USPQ2d 1962, 1966 (Fed. Cir. 1989) (The determination of whether preamble recitations are structural limitations can be resolved only on review of the entirety of the application "to gain an understanding of what the inventors actually invented and intended to encompass by the claim."). The absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. 112, para. 1, for lack of adequate written description. Limitations may not, however, be imported into the claims from the specification.

2. Review the Entire Application to Understand How Applicant Provides Support for the Claimed Invention Including Each Element and/or Step

Prior to determining whether the disclosure satisfies the written description requirement for the claimed subject matter, the examiner should review the claims and the entire specification, including the specific embodiments, figures, and sequence listings, to understand how applicant provides support for the various features of the claimed invention. An element may be critical where those of skill in the art would require it to determine that applicant was in possession of the invention. Compare *Rasmussen*, 650 F.2d at 1215, 211 USPQ at 327 ("one skilled in the art who read Rasmussen's specification would understand that it is unimportant how the layers are adhered, so long as they are adhered") (emphasis in original), with *Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) ("it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it"). The analysis of whether the specification complies with the written description requirement calls for the examiner to compare the scope of the claim with the scope of

the description to determine whether applicant has demonstrated possession of the claimed invention. Such a review is conducted from the standpoint of one of skill in the art at the time the application was filed (see, e.g., *Wang Labs. v. Toshiba Corp.*, 993 F.2d 858, 865, 26 USPQ2d 1767, 1774 (Fed. Cir. 1993)) and should include a determination of the field of the invention and the level of skill and knowledge in the art. Generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the specification. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986).

3. Determine Whether There is Sufficient Written Description to Inform a Skilled Artisan That Applicant was in Possession of the Claimed Invention as a Whole at the Time the Application Was Filed

(a) Original claims

Possession may be shown in many ways. For example, possession may be shown by describing an actual reduction to practice of the claimed invention. Possession may also be shown by a clear depiction of the invention in detailed drawings or in structural chemical formulas which permit a person skilled in the art to clearly recognize that applicant had possession of the claimed invention. An adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention. See, e.g., *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000) (the written description "inquiry is a factual one and must be assessed on a case-by-case basis"); see also *Pfaff v. Wells Electronics, Inc.*, 55 U.S. at 66, 119 S.Ct. at 311, 48 USPQ2d at 1646 ("The word 'invention' must refer to a concept that is complete, rather than merely one that is 'substantially complete.' It is true that reduction to practice ordinarily provides the best evidence that an invention is complete. But just because reduction to practice is sufficient evidence of completion, it does not follow that proof of reduction to practice is necessary in every case. Indeed, both the facts of the Telephone Cases and the facts of this case demonstrate that one can prove that an invention is complete and ready for patenting before it has actually been reduced to practice.").

A specification may describe an actual reduction to practice by showing that the inventor constructed an embodiment or performed a process that met all the limitations of the claim and determined that the invention would work for its intended purpose. *Cooper v. Goldfarb*, 154 F.3d 1321, 1327, 47 USPQ2d 1896, 1901 (Fed. Cir. 1998). See also *UMC Elecs. Co. v. United States*, 816 F.2d 647, 652, 2 USPQ2d 1465, 1468 (Fed. Cir. 1987) ("[T]here cannot be a reduction to practice of the invention *** without a physical embodiment which includes all limitations of the claim."); *Estee Lauder Inc. v. L'Oreal, S.A.*, 129 F.3d 588, 593, 44 USPQ2d 1610, 1614 (Fed. Cir. 1997) ("[A] reduction to practice does not occur until the inventor has determined that the invention will work for its intended purpose."); *Mahurkar v. C.R. Bard, Inc.*, 79 F.3d 1572, 1578, 38 USPQ2d 1288, 1291 (Fed. Cir. 1996) (determining that the invention will work for its intended purpose may require testing depending on the character of the invention and the problem it solves). Description of an actual reduction to practice of a biological material may be shown by specifically describing a deposit made in accordance with the requirements of 37 CFR 1.801 *et seq.* See especially 37 CFR 1.804 and 1.809. See also paragraph I.,

supra.

An applicant may show possession of an invention by disclosure of drawings or structural chemical formulas that are sufficiently detailed to show that applicant was in possession of the claimed invention as a whole. See, e.g., *Vas-Cath*, 935 F.2d at 1565, 19 USPQ2d at 1118 ("drawings alone may provide a 'written description' of an invention as required by Sec. 112*"); *In re Wolfensperger*, 302 F.2d 950, 133 USPQ 537 (CCPA 1962) (the drawings of applicant's specification provided sufficient written descriptive support for the claim limitation at issue); *Autogiro Co. of America v. United States*, 384 F.2d 391, 398, 155 USPQ 697, 703 (Ct. Cl. 1967) ("In those instances where a visual representation can flesh out words, drawings may be used in the same manner and with the same limitations as the specification."); *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 ("In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus."). The description need only describe in detail that which is new or not conventional. See *Hybritech v. Monoclonal Antibodies*, 802 F.2d at 1384, 231 USPQ at 94; *Fonar Corp. v. General Electric Co.*, 107 F.3d at 1549, 41 USPQ2d at 1805 (source code description not required). This is equally true whether the claimed invention is directed to a product or a process.

An applicant may also show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention, i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. *Enzo Biochem*, 323 F.3d at 964, 63 USPQ2d at 1613. For example, the presence of a restriction enzyme map of a gene may be relevant to a statement that the gene has been isolated. One skilled in the art may be able to determine whether the gene disclosed is the same as or different from a gene isolated by another by comparing the restriction enzyme maps. In contrast, evidence that the gene could be digested with a nuclease would not normally represent a relevant characteristic since any gene would be digested with a nuclease. Similarly, isolation of an mRNA and its expression to produce the protein of interest is strong evidence of possession of an mRNA for the protein.

For some biomolecules, examples of identifying characteristics include a sequence, structure, binding affinity, binding specificity, molecular weight, and length. Although structural formulas provide a convenient method of demonstrating possession of specific molecules, other identifying characteristics or combinations of characteristics may demonstrate the requisite possession. >As explained by the Federal Circuit, "(1) examples are not necessary to support the adequacy of a written description; (2) the written description standard may be met . even where actual reduction to practice of an invention is absent; and (3) there is no *per se* rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure." *Falkner v. Inglis*, 448 F.3d 1357, 1366, 79 USPQ2d 1001, 1007 (Fed. Cir. 2006). See also *Capon v. Eshhar*, 418 F.3d at 1358, 76 USPQ2d at 1084 ("The Board erred in holding that the specifications do not meet the written description requirement because they do not reiterate the structure or formula or chemical name for the nucleotide sequences of the claimed chimeric genes" where the genes were novel combinations of known DNA segments.).< For example, disclosure of an antigen

fully characterized by its structure, formula, chemical name, physical properties, or deposit in a public depository provides an adequate written description of an antibody claimed by its binding affinity to that antigen. *Noelle v. Lederman*, 355 F.3d 1343, 1349, 69 USPQ2d 1508, 1514 (Fed. Cir. 2004) (holding there is a lack of written descriptive support for an antibody defined by its binding affinity to an antigen that itself was not adequately described). Additionally, unique cleavage by particular enzymes, isoelectric points of fragments, detailed restriction enzyme maps, a comparison of enzymatic activities, or antibody cross-reactivity may be sufficient to show possession of the claimed invention to one of skill in the art. See *Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1966 ("written description" requirement may be satisfied by using "such descriptive means as words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention"). A definition by function alone "does not suffice" to sufficiently describe a coding sequence "because it is only an indication of what the gene does, rather than what it is." *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. See also *Fiers*, 984 F.2d at 1169-71, 25 USPQ2d at 1605-06 (discussing *Amgen Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991)). An adequate written description of a chemical invention also requires a precise definition, such as by structure, formula, chemical name, or physical properties, and not merely a wish or plan for obtaining the chemical invention claimed. See, e.g., *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 927, 69 USPQ2d 1886, 1894-95 (Fed. Cir. 2004) (The patent at issue claimed a method of selectively inhibiting PGHS-2 activity by administering a non-steroidal compound that selectively inhibits activity of the PGHS-2 gene product, however the patent did not disclose any compounds that can be used in the claimed methods. While there was a description of assays for screening compounds to identify those that inhibit the expression or activity of the PGHS-2 gene product, there was no disclosure of which peptides, polynucleotides, and small organic molecules selectively inhibit PGHS-2. The court held that "[w]ithout such disclosure, the claimed methods cannot be said to have been described.").

If a claim limitation invokes 35 U.S.C. 112, para. 6, it must be interpreted to cover the corresponding structure, materials, or acts in the specification and "equivalents thereof." See 35 U.S.C. 112, para. 6. See also *B. Braun Medical, Inc. v. Abbott Lab.*, 124 F.3d 1419, 1424, 43 USPQ2d 1896, 1899 (Fed. Cir. 1997). In considering whether there is 35 U.S.C. 112, para. 1, support for a means- (or step) plus-function claim limitation, the examiner must consider not only the original disclosure contained in the summary and detailed description of the invention portions of the specification, but also the original claims, abstract, and drawings. A means- (or step-) plus-function claim limitation is adequately described under 35 U.S.C. 112, para. 1, if: (1) The written description adequately links or associates adequately described particular structure, material, or acts to the function recited in a means- (or step-) plus-function claim limitation; or (2) it is clear based on the facts of the application that one skilled in the art would have known what structure, material, or acts perform the function recited in a means- (or step-) plus-function limitation. Note also: A rejection under 35 U.S.C. 112, para. 2, "cannot stand where there is adequate description in the specification to satisfy 35 U.S.C. 112, first paragraph, regarding means-plus-function recitations that are not, *per se*, challenged for being unclear." *In re Noll*, 545 F.2d 141, 149, 191 USPQ 721, 727 (CCPA 1976). See Supplemental Examination Guidelines for Determining the Applicability of 35 U.S.C. 112, para. 6, 65 Fed. Reg. 38510, June 21, 2000. See also MPEP § 2181.

What is conventional or well known to one of ordinary skill in the art need not be

disclosed in detail. See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231 USPQ at 94. >See also *Capon v. Eshhar*, 418 F.3d 1349, 1357, 76 USPQ2d 1078, 1085 (Fed. Cir. 2005) ("The 'written description' requirement must be applied in the context of the particular invention and the state of the knowledge.. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution.").< If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met. See, e.g., *Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116; *Martin v. Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (stating "the description need not be in *ipsis verbis* [i.e., "in the same words"] to be sufficient").

A claim which is limited to a single disclosed embodiment or species is analyzed as a claim drawn to a single embodiment or species, whereas a claim which encompasses two or more embodiments or species within the scope of the claim is analyzed as a claim drawn to a genus. See also MPEP § 806.04(e).

For Each Claim Drawn to a Single Embodiment or Species:

- (A) Determine whether the application describes an actual reduction to practice of the claimed invention.
- (B) If the application does not describe an actual reduction to practice, determine whether the invention is complete as evidenced by a reduction to drawings or structural chemical formulas that are sufficiently detailed to show that applicant was in possession of the claimed invention as a whole.
- (C) If the application does not describe an actual reduction to practice or reduction to drawings or structural chemical formula as discussed above, determine whether the invention has been set forth in terms of distinguishing identifying characteristics as evidenced by other descriptions of the invention that are sufficiently detailed to show that applicant was in possession of the claimed invention.
 - (1) Determine whether the application as filed describes the complete structure (or acts of a process) of the claimed invention as a whole. The complete structure of a species or embodiment typically satisfies the requirement that the description be set forth "in such full, clear, concise, and exact terms" to show possession of the claimed invention. 35 U.S.C. 112, para. 1. Cf. *Fields v. Conover*, 443 F.2d 1386, 1392, 170 USPQ 276, 280 (CCPA 1971) (finding a lack of written description because the specification lacked the "full, clear, concise, and exact written description" which is necessary to support the claimed invention). If a complete structure is disclosed, the written description requirement is satisfied for that species or embodiment, and a rejection under 35 U.S.C. 112, para. 1, for lack of written description must not be made.
 - (2) If the application as filed does not disclose the complete structure (or acts of a process) of the claimed invention as a whole, determine whether the specification discloses other relevant identifying characteristics sufficient to describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention. For example, if the art has established a strong correlation between structure and function, one skilled in the art would be able to predict with a reasonable degree of confidence the structure of the

claimed invention from a recitation of its function. Thus, the written description requirement may be satisfied through disclosure of function and minimal structure when there is a well-established correlation between structure and function. In contrast, without such a correlation, the capability to recognize or understand the structure from the mere recitation of function and minimal structure is highly unlikely. In this latter case, disclosure of function alone is little more than a wish for possession; it does not satisfy the written description requirement. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 (written description requirement not satisfied by merely providing "a result that one might achieve if one made that invention"); *In re Wilder*, 736 F.2d 1516, 1521, 222 USPQ 369, 372-73 (Fed. Cir. 1984) (affirming a rejection for lack of written description because the specification does "little more than outline goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate"). Compare *Fonar*, 107 F.3d at 1549, 41 USPQ2d at 1805 (disclosure of software function adequate in that art).

Whether the specification shows that applicant was in possession of the claimed invention is not a single, simple determination, but rather is a factual determination reached by considering a number of factors. Factors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

>The description needed to satisfy the requirements of 35 U.S.C. 112 "varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence." *Capon v. Eshhar*, 418 F.3d at 1357, 76 USPQ2d at 1084.< Patents and printed publications in the art should be relied upon to determine whether an art is mature and what the level of knowledge and skill is in the art. In most technologies which are mature, and wherein the knowledge and level of skill in the art is high, a written description question should not be raised for * claims >present in the application when originally filed,< even if the specification discloses only a method of making the invention and the function of the invention. See, e.g., *In re Hayes Microcomputer Products, Inc. Patent Litigation*, 982 F.2d 1527, 1534-35, 25 USPQ2d 1241, 1246 (Fed. Cir. 1992) ("One skilled in the art would know how to program a microprocessor to perform the necessary steps described in the specification. Thus, an inventor is not required to describe every detail of his invention. An applicant's disclosure obligation varies according to the art to which the invention pertains. Disclosing a microprocessor capable of performing certain functions is sufficient to satisfy the requirement of section 112, first paragraph, when one skilled in the relevant art would understand what is intended and know how to carry it out.").

In contrast, for inventions in emerging and unpredictable technologies, or for inventions characterized by factors not reasonably predictable which are known to one of ordinary skill in the art, more evidence is required to show possession. For example, disclosure of only a method of making the invention and the function may not be sufficient to support a product claim other than a product-by-process claim. See, e.g., *Fiers v. Revel*, 984 F.2d at 1169, 25 USPQ2d at 1605; *Amgen*, 927 F.2d at 1206, 18 USPQ2d at 1021. Where the process has actually been used to produce the product, the written description requirement for a product-by-process claim is clearly satisfied; however, the requirement may not be satisfied where it is not clear that the acts set forth in the specification can be

performed, or that the product is produced by that process. Furthermore, disclosure of a partial structure without additional characterization of the product may not be sufficient to evidence possession of the claimed invention. See, e.g., *Amgen*, 927 F.2d at 1206, 18 USPQ2d at 1021 ("A gene is a chemical compound, albeit a complex one, and it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it. Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. It is not sufficient to define it solely by its principal biological property, e.g., encoding human erythropoietin, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property. We hold that when an inventor is unable to envision the detailed constitution of a gene so as to distinguish it from other materials, as well as a method for obtaining it, conception has not been achieved until reduction to practice has occurred, i.e., until after the gene has been isolated.") (citations omitted). In such instances the alleged conception fails not merely because the field is unpredictable or because of the general uncertainty surrounding experimental sciences, but because the conception is incomplete due to factual uncertainty that undermines the specificity of the inventor's idea of the invention. *Burroughs Wellcome Co. v. Barr Laboratories Inc.*, 40 F.3d 1223, 1229, 32 USPQ2d 1915, 1920 (Fed. Cir. 1994). Reduction to practice in effect provides the only evidence to corroborate conception (and therefore possession) of the invention. *Id.*

Any claim to a species that does not meet the test described under at least one of (a), (b), or (c) must be rejected as lacking adequate written description under 35 U.S.C. 112, para. 1.

For each claim drawn to a genus:

The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice (see i)(A), above), reduction to drawings (see i)(B), above), or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (see i)(C), above). See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. The disclosure of only one species encompassed within a genus adequately describes a claim directed to that genus only if the disclosure "indicates that the patentee has invented species sufficient to constitute the gen[us]." See *Enzo Biochem*, 323 F.3d at 966, 63 USPQ2d at 1615; *Noelle v. Lederman*, 355 F.3d 1343, 1350, 69 USPQ2d 1508, 1514 (Fed. Cir. 2004) (Fed. Cir. 2004)("[A] patentee of a biotechnological invention cannot necessarily claim a genus after only describing a limited number of species because there may be unpredictability in the results obtained from species other than those specifically enumerated."). "A patentee will not be deemed to have invented species sufficient to constitute the genus by virtue of having disclosed a single species when . the evidence indicates ordinary artisans could not predict the

operability in the invention of any species other than the one disclosed." *In re Curtis*, 354 F.3d 1347, 1358, 69 USPQ2d 1274, 1282 (Fed. Cir. 2004)(Claims directed to PTFE dental floss with a friction-enhancing coating were not supported by a disclosure of a microcrystalline wax coating where there was no evidence in the disclosure or anywhere else in the record showing applicant conveyed that any other coating was suitable for a PTFE dental floss.) On the other hand, there may be situations where one species adequately supports a genus. See, e.g., *Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326-27 (disclosure of a single method of adheringly applying one layer to another was sufficient to support a generic claim to "adheringly applying" because one skilled in the art reading the specification would understand that it is unimportant how the layers are adhered, so long as they are adhered); *In re Herschler*, 591 F.2d 693, 697, 200 USPQ 711, 714 (CCPA 1979) (disclosure of corticosteroid in DMSO sufficient to support claims drawn to a method of using a mixture of a "physiologically active steroid" and DMSO because "use of known chemical compounds in a manner auxiliary to the invention must have a corresponding written description only so specific as to lead one having ordinary skill in the art to that class of compounds. Occasionally, a functional recitation of those known compounds in the specification may be sufficient as that description."); *In re Smythe*, 480 F.2d 1376, 1383, 178 USPQ 279, 285 (CCPA 1973) (the phrase "air or other gas which is inert to the liquid" was sufficient to support a claim to "inert fluid media" because the description of the properties and functions of the air or other gas segmentizing medium would suggest to a person skilled in the art that appellant's invention includes the use of "inert fluid" broadly.).

**>The Federal Circuit has explained that a specification cannot always support expansive claim language and satisfy the requirements of 35 U.S.C. 112 "merely by clearly describing one embodiment of the thing claimed." *LizardTech v. Earth Resource Mapping, Inc.*, 424 F.3d 1336, 1346, 76 USPQ2d 1731, 1733 (Fed. Cir. 2005). The issue is whether a person skilled in the art would understand applicant to have invented, and been in possession of, the invention as broadly claimed. In *LizardTech*, claims to a generic method of making a seamless discrete wavelet transformation (DWT) were held invalid under 35 U.S.C. 112, first paragraph because the specification taught only one particular method for making a seamless DWT and there was no evidence that the specification contemplated a more generic method. See also< *Tronzo v. Biomet*, 156 F.3d at 1159, 47 USPQ2d at 1833 (Fed. Cir. 1998), >wherein< the disclosure of a species in the parent application did not suffice to provide written description support for the genus in the child application.

What constitutes a "representative number" is an inverse function of the skill and knowledge in the art. Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus. See, e.g., *Eli Lilly*. Description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces. For example, in the molecular biology arts, if an applicant disclosed an amino acid sequence, it would be unnecessary to provide an explicit disclosure of nucleic acid sequences that encoded the amino acid sequence. Since the genetic code is widely known, a disclosure of an amino acid sequence would provide sufficient information such that one would accept that an applicant was in possession of the full genus of nucleic

acids encoding a given amino acid sequence, but not necessarily any particular species. Cf. *In re Bell*, 991 F.2d 781, 785, 26 USPQ2d 1529, 1532 (Fed. Cir. 1993) and *In re Baird*, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994). If a representative number of adequately described species are not disclosed for a genus, the claim to that genus must be rejected as lacking adequate written description under 35 U.S.C. 112, para. 1.

(b) New Claims, Amended Claims, or Claims Asserting Entitlement to the Benefit of an Earlier Priority Date or Filing Date under 35 U.S.C. 119, 120, or 365(c)

The examiner has the initial burden of presenting evidence or reasoning to explain why persons skilled in the art would not recognize in the original disclosure a description of the invention defined by the claims. See *Wertheim*, 541 F.2d at 263, 191 USPQ at 97 ("[T]he PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims."). However, when filing an amendment an applicant should show support in the original disclosure for new or amended claims. See MPEP § 714.02 and § 2163.06 ("Applicant should *** specifically point out the support for any amendments made to the disclosure.").

To comply with the written description requirement of 35 U.S.C. 112, para. 1, or to be entitled to an earlier priority date or filing date under 35 U.S.C. 119, 120, or 365(c), each claim limitation must be expressly, implicitly, or inherently supported in the originally filed disclosure. When an explicit limitation in a claim "is not present in the written description whose benefit is sought it must be shown that a person of ordinary skill would have understood, at the time the patent application was filed, that the description requires that limitation." *Hyatt v. Boone*, 146 F.3d 1348, 1353, 47 USPQ2d 1128, 1131 (Fed. Cir. 1998). See also *In re Wright*, 866 F.2d 422, 425, 9 USPQ2d 1649, 1651 (Fed. Cir. 1989) (Original specification for method of forming images using photosensitive microcapsules which describes removal of microcapsules from surface and warns that capsules not be disturbed prior to formation of image, unequivocally teaches absence of permanently fixed microcapsules and supports amended language of claims requiring that microcapsules be "not permanently fixed" to underlying surface, and therefore meets description requirement of 35 U.S.C. 112.); *In re Robins*, 429 F.2d 452, 456-57, 166 USPQ 552, 555 (CCPA 1970) ("[W]here no explicit description of a generic invention is to be found in the specification[,] ... mention of representative compounds may provide an implicit description upon which to base generic claim language."); *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) (a subgenus is not necessarily implicitly described by a genus encompassing it and a species upon which it reads); *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) ("To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.'") (citations omitted). Furthermore, each claim must include all elements which applicant has described as essential. See, e.g., *Johnson Worldwide Associates Inc. v. Zebco Corp.*, 175 F.3d at 993, 50 USPQ2d at 1613; *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d at 1479, 45 USPQ2d at 1503; *Tronzo v. Biomet*, 156 F.3d at 1159, 47 USPQ2d at 1833.

If the originally filed disclosure does not provide support for each claim limitation, or if an element which applicant describes as essential or critical is not claimed, a new or

amended claim must be rejected under 35 U.S.C. 112, para. 1, as lacking adequate written description, or in the case of a claim for priority under 35 U.S.C. 119, 120, or 365(c), the claim for priority must be denied.

III. COMPLETE PATENTABILITY DETERMINATION UNDER ALL STATUTORY REQUIREMENTS AND CLEARLY COMMUNICATE FINDINGS, CONCLUSIONS, AND THEIR BASES

The above only describes how to determine whether the written description requirement of 35 U.S.C. 112, para. 1, is satisfied. Regardless of the outcome of that determination, Office personnel must complete the patentability determination under all the relevant statutory provisions of title 35 of the U.S. Code.

Once Office personnel have concluded analysis of the claimed invention under all the statutory provisions, including 35 U.S.C. 101, 112, 102, and 103, they should review all the proposed rejections and their bases to confirm their correctness. Only then should any rejection be imposed in an Office action. The Office action should clearly communicate the findings, conclusions, and reasons which support them. When possible, the Office action should offer helpful suggestions on how to overcome rejections.

A. For Each Claim Lacking Written Description Support, Reject the Claim Under 35 U.S.C. 112, para. 1, for Lack of Adequate Written Description

A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. See, e.g., *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. *Wertheim*, 541 F.2d at 263, 191 USPQ at 97. In rejecting a claim, the examiner must set forth express findings of fact regarding the above analysis which support the lack of written description conclusion. These findings should:

(A) Identify the claim limitation at issue; and

(B) Establish a *prima facie* case by providing reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed. A general allegation of "unpredictability in the art" is not a sufficient reason to support a rejection for lack of adequate written description.

When appropriate, suggest amendments to the claims which can be supported by the application's written description, being mindful of the prohibition against the addition of new matter in the claims or description. See *Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326.

B. Upon Reply by Applicant, Again Determine the Patentability of the Claimed Invention, Including Whether the Written Description Requirement Is Satisfied by Reperforming the Analysis Described Above in View of the Whole Record

Upon reply by applicant, before repeating any rejection under 35 U.S.C. 112, para. 1, for lack of written description, review the basis for the rejection in view of the record as a whole, including amendments, arguments, and any evidence submitted by applicant. If the whole record now demonstrates that the written description requirement is satisfied, do not repeat the rejection in the next Office action. If the record still does not demonstrate that the written description is adequate to support the claim(s), repeat the rejection under 35 U.S.C. 112, para. 1, fully respond to applicant's rebuttal arguments, and properly treat any further showings submitted by applicant in the reply. When a rejection is maintained, any affidavits relevant to the 112, para. 1, written description requirement, must be thoroughly analyzed and discussed in the next Office action. See *In re Alton*, 76 F.3d 1168, 1176, 37 USPQ2d 1578, 1584 (Fed. Cir. 1996).

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2164.04 Burden on the Examiner Under *>the< Enablement Requirement [R-1] - 2100 Patentability

2164.04 Burden on the Examiner Under *>the< Enablement Requirement [R-1]

Before any analysis of enablement can occur, it is necessary for the examiner to construe the claims. For terms that are not well-known in the art, or for terms that could have more than one meaning, it is necessary that the examiner select the definition that he/she intends to use when examining the application, based on his/her understanding of what applicant intends it to mean, and explicitly set forth the meaning of the term and the scope of the claim when writing an Office action. See *Genentech v. Wellcome Foundation*, 29 F.3d 1555, 1563-64, 31 USPQ2d 1161, 1167-68 (Fed. Cir. 1994).

In order to make a rejection, the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993) (examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure). A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. Assuming that sufficient reason for such doubt exists, a rejection for failure to teach how to make and/or use will be proper on that basis. *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). As stated by the court, "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." 439 F.2d at 224, 169 USPQ at 370.

According to *In re Bowen*, 492 F.2d 859, 862-63, 181 USPQ 48, 51 (CCPA 1974), the minimal requirement is for the examiner to give reasons for the uncertainty of the enablement. This standard is applicable even when there is no evidence in the record of operability without undue experimentation beyond the disclosed embodiments. See also *In re Brana*, 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995) (citing *In re*

Bundy, 642 F.2d 430, 433, 209 USPQ 48, 51 (CCPA 1981) (discussed in MPEP § 2164.07 regarding the relationship of the enablement requirement to the utility requirement of 35 U.S.C. 101).

While the analysis and conclusion of a lack of enablement are based on the factors discussed in MPEP § 2164.01(a) and the evidence as a whole, it is not necessary to discuss each factor in the written enablement rejection. The language should focus on those factors, reasons, and evidence that lead the examiner to conclude that the specification fails to teach how to make and use the claimed invention without undue experimentation, or that the scope of any enablement provided to one skilled in the art is not commensurate with the scope of protection sought by the claims. This can be done by making specific findings of fact, supported by the evidence, and then drawing conclusions based on these findings of fact. For example, doubt may arise about enablement because information is missing about one or more essential parts or relationships between parts which one skilled in the art could not develop without undue experimentation. In such a case, the examiner should specifically identify what information is missing and why one skilled in the art could not supply the information without undue experimentation. See MPEP § 2164.06(a). References should be supplied if possible to support a *prima facie* case of lack of enablement, but are not always required. *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). However, specific technical reasons are always required.

In accordance with the principles of compact prosecution, if an enablement rejection is appropriate, the first Office action on the merits should present the best case with all the relevant reasons, issues, and evidence so that all such rejections can be withdrawn if applicant provides appropriate convincing arguments and/or evidence in rebuttal.

Providing the best case in the first Office action will also allow the second Office action to be made final should applicant fail to provide appropriate convincing arguments and/or evidence. Citing new references and/or expanding arguments in a second Office action could prevent that Office action from being made final. The principles of compact prosecution also dictate that if an enablement rejection is appropriate and the examiner recognizes limitations that would render the claims enabled, the examiner should note such limitations to applicant as early in the prosecution as possible.

In other words, the examiner should always look for enabled, allowable subject matter and communicate to applicant what that subject matter is at the earliest point possible in the prosecution of the application.

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The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

Paper No. 76

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte KENNETH F. BUECHLER,
RICHARD R. ANDERSON,
THEODORE T. LEE, and
GUNARS E. VALKIRS

Appeal No. 2003-2084
Application No. 08/241,061

HEARD: October 7, 2003

WILLIAM F. SMITH, MILLS, and GRIMES, Administrative Patent Judges.

WILLIAM F. SMITH, Administrative Patent Judge.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 from the final rejection of claims 98 through 108. Claims 98 and 99 are representative of the subject matter on appeal and read as follows:

98. A composition of matter comprising:

(a) a plurality of different ligand analogue conjugates, each different ligand analogue conjugate corresponding to one of a plurality of different target ligands, wherein each ligand analogue conjugate comprises a binding site for an antibody or a ligand receptor produced by a standard immunologic technique, and wherein each ligand analogue conjugate is attached via a linkage site to a protein, polypeptide, polymer, or a signal

development element, said linkage site being the same or different from the linkage site of the other ligand analogue conjugates; and

(b) at least one crosstalk inhibitor comprising an analogue of at least one linkage site present amongst the plurality of different ligand analogue conjugates, wherein the crosstalk inhibitor is present in an amount sufficient to inhibit binding of the linkage site of at least one ligand analogue conjugate in a ligand receptor assay to an antibody or ligand receptor produced by a standard immunologic technique.

99. A composition for use in an assay for determining the amount or presence of a target ligand, wherein the target ligand is a binding partner to a ligand receptor, the composition comprising:

(a) a ligand analogue conjugate comprising a binding site for a receptor attached via a linkage site to a protein, polypeptide, polymer, or signal development element;

(b) a ligand receptor comprising an antibody or a fragment thereof which specifically binds the ligand analogue conjugate; and

(c) a crosstalk inhibitor comprising at least one analogue of the linkage site in an amount sufficient to inhibit binding of the ligand receptor to the linkage site.

The references relied upon by the examiner are:

Schuurs et al. (Schuurs) 3,879,262 Apr. 22, 1975

Kinoshita et al. (Kinoshita), "Enzyme Immunoassay for Captopril," Journal of Pharmaceutical Sciences, Vol. 75, No. 7, pp. 711-713 (1986)

Marini et al. (Marini), "A Simple Method for Increasing Hapten Immunogenicity by a Specific Structural Modification of the Carrier," Journal of Immunological Methods, Vol. 120, pp. 57-63 (1989)

The claims on appeal stand rejected as follows:

Claim 102 under 35 U.S.C. § 112, first paragraph (written description);

Claims 98 and 99 under 35 U.S.C. § 112, first paragraph (written description);

Claims 98-102 under 35 U.S.C. § 112, first paragraph (enablement);

Claims 98-108 under 35 U.S.C. § 112, second paragraph, as being indefinite;
Claims 98-102 under 35 U.S.C. § 102(b) as anticipated by Kinoshita;

Claims 99-102 under 35 U.S.C. § 102(b) as anticipated by Marini; and,

Claims 99-102 under 35 U.S.C. § 102(b) as anticipated by Schuurs.

We reverse all rejections.

Background

The present invention is concerned with a problem in the immunoassay art known as crosstalk. As explained:

Methods to prepare monoclonal antibodies to ligands which, by themselves, do not generate an immunological response are well known to those skilled in the art. The ligand, or an analogue thereof, is generally coupled, chemically, to a carrier molecule, e.g., a protein, peptide, or other polymer, to form an immunogen (one example of a ligand analogue conjugate as defined herein) which elicits an immunological response. Antibodies are thus raised to the surface of the carrier molecule onto which is coupled the ligand. The selection or screening of antibodies is then performed to choose the antibody which best fulfills the intended use of the antibody. The screening of antibodies is well known to those skilled in the art and is generally performed by binding a ligand-carrier conjugate to a solid phase, allowing the raised antibody to bind to the ligand-carrier conjugate and detecting the presence of the bound antibody with a labelled anti-antibody conjugate. An inherent problem with the generation and screening of antibodies is the difficulty in determining the location of binding of the antibody to the ligand, i.e., the binding site; that is, it is not clear which portion of the ligand analogue is bound by the antibody. This can result in the selection of antibodies which possess a very small but definite affinity to the carrier molecule, or to the chemical structure (herein called the "linkage site") which attaches the ligand analogue to carrier molecule. Such an antibody, will thus bind (an occurrence known as crosstalk) to other, or uncomplementary, carrier molecule-ligand complexes having such a linkage, and produce false positive results when such other complexes are present in a test.

Specification, page 6.

The crosstalk problem is addressed by the present invention as follows:

The present invention is directed to ligand receptor assays in which the presence of a multiplicity of ligands are measured in a single determination. In particular, the present invention relates to the preparation and use of reagents as crosstalk inhibitors in ligand receptor assays. The crosstalk inhibitors resemble the chemical structure (or linkage site) which links the ligand analogue to the carrier molecule of a ligand analogue conjugate. Thus, the crosstalk inhibitors reduce or

prevent the crosstalk, i.e., the undesirable interactions between ligand receptors and uncomplementary ligand analogue conjugates.

Specification, page 8.

The crosstalk inhibitors of the present invention are described as follows:

The crosstalk inhibitor, which resembles the linkage chemistry of the ligand analogue conjugates, competes with the linkage chemistry of the ligand analogue conjugates for binding to the terminal solid phase ligand receptor. With the proper crosstalk inhibitor and crosstalk inhibitor concentration, the competition is shifted toward binding of the crosstalk inhibitor and not of the uncomplementary ligand analogue conjugates. The crosstalk inhibitor should compete very poorly with the complementary ligand analogue conjugate for the solid phase ligand receptor because the affinity of the ligand receptor for the complementary ligand analogue conjugate is much higher.

How closely the chemical structure of the crosstalk inhibitor must resemble the linkage chemistry of the ligand analogue depends on the affinity of the ligand receptor for the uncomplementary ligand analogue conjugate. The crosstalk inhibitor may be free in solution or bound to a protein or polymer. When the crosstalk inhibitor is attached to a protein or polymer, it can bind multivalently to the solid phase ligand receptor as can the ligand analogue conjugate. Thus, the multivalent crosstalk inhibitor can better compete with the uncomplementary ligand analogue conjugate than the monovalent crosstalk inhibitor.

Specification, pages 9-10.

As seen from claims 98 and 99, the claimed invention is directed to a composition that comprises a ligand analogue conjugate(s), and a crosstalk inhibitor with or without a ligand receptor. Ligand analogue conjugate is defined as “[a] conjugate of a ligand analogue and a signal development element, a protein, polypeptide, or polymer.” Specification, page 11, lines 14-22.

Discussion

1. Written Description Rejection of Claim 102.

The examiner explains the rejection as follows:

The ligand is 'drug of abuse, metabolite of drug abuse, an analogue of the drug abuse, an analogue of the metabolite of the drug abuse, therapeutic drug, a metabolite of a therapeutic drug, an analogue of a therapeutic drug, and an analogue of a metabolite of a therapeutic drug...' claimed in claim 102 has no clear support in the specification and the claims as originally filed. The subject matter claimed in claims [sic] 102 broadens the scope of the invention as originally disclosed in the specification.

Examiner's Answer, page 4.

Appellants argue that literal support for the language is found in the specification at page 1, line 10 through page 2, line 16 and page 11, lines 9-13. Appeal Brief, pages 28-30. The examiner states that appellants may not rely upon that portion of the specification at page 1 since that portion is "Background of the Invention" and not "drawn to the composition as claimed in the instant claims." Examiner's Answer, page 12.

In reviewing the matter, we find ourselves in agreement with appellants. The examiner is correct in pointing out that the portion of the specification at page 1 which appellants rely upon for written descriptive support of the questioned claim language is headed by the title "Background of the Invention." However the specific text relied upon by appellants uses phrases such as "as used herein" and "in the context of the present invention." Clearly, in describing the "Background of the Invention," appellants are setting forth part of the present invention. In other words, the present invention, as do most inventions, builds upon what was known in the art. The present specification

provides adequate written descriptive support for the language questioned by the examiner.

If the examiner's real concern is that the language set forth in claim 102 does not appear verbatim in the specification, the examiner should require appellants to comply with 37 CFR § 1.75(d)(1) ("The claim or claims must conform to the invention as set forth in the remainder of the specification and the terms and phrases used in the claims must find clear support or antecedent basis in the description so that the meaning of the terms in the claims may be ascertainable by reference to the description.")

The rejection of claim 102 under 35 U.S.C. § 112, first paragraph (written description), is reversed.

2. Written Description Rejection of Claims 98 and 99.

The rejection is explained as follows:

The specification description directed [sic] is directed to specific crosstalk inhibitors which resemble the chemical structure which links the ligand analogue to the carrier, for example the crosstalk inhibitors disclosed in figures 1C to 1F, which clearly do not provide an adequate representation regarding the open ended claimed composition comprising the crosstalk inhibitors, ligand analogue conjugates attached to a protein made of the presently claimed invention.

And moreover, applicants have not shown that they are in possession of a composition which has plurality of different ligand analogue conjugates, each different ligand analogue conjugate has a different linkage site from the linkage of the other ligand analogue conjugates.

Examiner's Answer, page 5.

The examiner relies upon the University of Calif. v. Eli Lilly & Co., 119 F.3d 1559, 1567, 43 USPQ2d 1398, 1405 (Fed. Cir. 1997), stating "[a]lthough directed to DNA compounds, this holding would be deemed to be applicable to any compound; which

requires a representative sample of compounds and/or a showing of sufficient identifying characteristics; to demonstrate possession of the claimed generic(s)."

Examiner's Answer, page 6.

Appellants argue, inter alia:

Moreover, Appellants respectfully submit that the specification as filed clearly indicates to the skilled artisan that Appellants were in possession of the claimed invention at the time of filing. The specification describes the common structural attributes shared by crosstalk inhibitors as defined in the instant specification (i.e., that each is an analogue of a linkage site), as well as common functional attributes shared by crosstalk inhibitors (i.e., that each inhibits crosstalk caused by receptors that recognize the linkage chemistry rather than the ligand). The specification follows this general description of the common structural and functional attributes of crosstalk inhibitors by describing the synthesis of numerous specific crosstalk inhibitors (see, e.g., examples 4-13 and 22). Finally, the specification also describes methods for testing the effectiveness of crosstalk inhibitors in ligand-receptor assays (see, e.g., example 30).

Appeal Brief, paragraph bridging pages 39-40. In response, the examiner states:

Appellants [sic] arguments regarding the possession of the claimed composition at the time of filing have been considered, but are not persuasive. Appellants argue that the instant claimed composition comprise a mixture of ligand analogue conjugates, cross talk inhibitors and ligand receptors. It is noted that claim 98 composition does not have the ligand receptor as in appellants [sic] argument. Appellants assert that the specification describes the common structural attributes shared by cross talk [sic] inhibitors as defined, as well as common functional attributes shared by cross talk [sic] inhibitors. Appellants [sic] assertions have been considered but are not persuasive. The narrow scope of examples directed to specific crosstalk inhibitors are clearly not representative of the scope of the presently claimed composition.

Examiner's Answer, page 13, 2nd paragraph (emphasis in original).

In considering the matter, we find ourselves in agreement with appellants' position again. Appellants have carefully explained how the specification reasonably describes a genus of crosstalk inhibitors. The examiner has focused upon the so-called "open ended claimed composition" (Examiner's Answer, page 5) and "narrow scope of

examples directed to specific crosstalk inhibitors" (Examiner's Answer, page 13)(emphasis in original). Merely pointing to the breadth of a claim limitation does not establish that that claim limitation does not enjoy written descriptive support as required by 35 U.S.C. § 112, first paragraph. On this record, we do not find that the examiner has established a prima facie case of lack of written description for claims 98 and 99.

The rejection under 35 U.S.C. § 112, first paragraph (written description) of claims 98 and 99 is reversed.

3. Enablement Rejection of Claims 98-102.

The examiner considers the specification to be enabling for compositions "comprising specific crosstalk Inhibitors (as in figures 1C-1F) and ligand conjugate, [sic]" but not for "any composition comprising ligand analogue conjugate and any crosstalk inhibitors." Examiner's Answer, page 6. In support of the rejection, the examiner provides an analysis of the factors set forth in In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988), i.e., (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

We first point out that application 07/683,456 is stated to be a parent application of this application. U.S. Patent No. 5,525,524 ('524 patent) also traces its parentage to application 07/683,456. In response to an obviousness-type double patenting rejection based upon the claims of the '524 patent, appellants have filed a terminal disclaimer.

Paper No. 43, April 23, 1999. Claim 3 of the '524 patent reads as follows:

3. A method for identifying a crosstalk inhibitor for a ligand analogue conjugate, wherein said ligand analogue conjugate comprises a binding site attached via a linkage site to a protein, polypeptide or label, and wherein said crosstalk inhibitor competes with said linkage site for binding to a ligand receptor, wherein said ligand receptor is an antibody which binds said linkage site, said method comprising the steps of:

(a) making a potential crosstalk inhibitor comprising at least one analogue of a linkage site of a ligand analogue conjugate,

(b) testing said potential crosstalk inhibitor by:

(i) performing a first assay utilizing a first reaction mixture comprising said ligand analogue conjugate and a ligand receptor, wherein said ligand receptor binds said linkage site causing a false positive result;

(ii) performing a second assay utilizing a second reaction mixture comprising said ligand analogue conjugate, said potential crosstalk inhibitor and said ligand receptor;

(iii) comparing the results of said first assay and said second assay, so that no false positive result or a reduced false positive result in said second assay indicates that said potential crosstalk inhibitor is useful crosstalk inhibitor.

As seen from claim 3 of the '524 patent, the USPTO has already determined that a method for identifying crosstalk inhibitors of the present invention is patentable subject matter. The present enablement rejection questions whether one skilled in the art would be able to identify crosstalk inhibitors without undue experimentation. It appears that the USPTO has already answered that question in the affirmative by issuing claim 3 of the '524 patent.

Furthermore, the examiner's analysis does not take into account the starting point of a crosstalk inhibitor, i.e., knowledge of the linkage site which is part of the ligand analogue conjugate. Development of a crosstalk inhibitor of the present invention does not begin in a vacuum. Rather, the skilled artisan must start with

knowledge of the linkage site of the ligand analogue conjugate in order to develop analogues to the linkage site to evaluate as possible crosstalk inhibitors.

The examiner's evaluation of the Wands factors overemphasizes the examiner's view of the breadth of the claims and does not take into account that the USPTO has allowed claim 3 of the '524 patent as well as the fact that the starting point of the present invention includes knowledge of the linkage site of the ligand analogue conjugate.

The rejection of claims 98-102 under 35 U.S.C. § 112, first paragraph (enablement), is reversed.

4. Rejection of claims 98-108 Under 35 U.S.C. § 112, second paragraph.

The examiner has set forth six reasons why the claims on appeal are indefinite on pages 9-10 of the Examiner's Answer. Included are questioning of the phrase "corresponding to" as it appears in claims 98-108 and the phrase "standard immunological technique" as it appears in claim 98.¹ However, the examiner states at page 3 of the Examiner's Answer, "[t]he rejection of claims 103-108 'corresponding to'; and the rejection of claim 99 'standard immunological technique' under 35 U.S.C. 112, second paragraph as indefinite has been withdrawn." Presumably the examiner withdrew the rejection of claim 99 in regard to the phrase "standard immunological technique" because the language does not appear in the claim. As the record stands, the examiner on one hand withdraws the rejection in this regard and on the other hand maintains the rejection. Given the examiner's positive statement that the rejection has

¹ The examiner states at page 9 of the answer that claim 99 also contains this language. However, the record copy of claim 99 (Paper No. 50) does not contain the phrase "standard immunological technique."

been withdrawn as to these two claim limitations, we conclude that the continued maintenance of this aspect of the rejection is an oversight on the part of the examiner.

We will also reverse the remainder of the rejection. The examiner first questions the phrase "ligand analogue conjugate" as used in claims 98-108 stating "[i]t is not clear what does [sic] applicants mean by analogue." However, as set forth above, the specification provides an explicit definition of "ligand analogue conjugate." Furthermore, this aspect of the rejection is contrary to the issuance of the '524 patent by the USPTO since claims of that patent are also directed to ligand analogue conjugates.

The examiner next questions the phrase "analogue of linkage site" as used in claims 98-99, stating "[t]he specification no where teaches what are the analogues of the linkage site. And the specification no where teaches how are the analogues in the linkage site can be [sic] prepared." Examiner's Answer, page 9. The specific questions raised by the examiner in regard to this claim language appear to be more directed to enablement rather than exploring the metes and bounds of the claim language. The examiner has not established that one skilled in the art would have any difficulty in determining whether a given compound would be considered an "analogue of the linkage site" as this phrase is used in claims 98 and 99. Again, this aspect of the rejection is in conflict with the issuance of the '524 patent by the USPTO since the phrase "analogue of the linkage site" is used in claim 3 of the '524 patent.

The next aspect of the rejection is the use of the phrase "standard immunological technique" in claim 98 with the examiner stating: "it is not clear what does [sic] applicants mean by 'standard immunological techniques[']. Does applicant [sic] mean

that the analogue is produced by the standard immunological techniques, it is not clear what are the the standard immunological techniques. The specification no where teaches the' standard immunological techniques['] useful in the claimed invention." Examiner's Answer, pages 9-10.

Appellants respond that the phrase refers to ligand receptors and "one of skill in the art would understand that ligand receptors produced by 'standard immunological techniques' refers to methods or techniques used to prepare antibodies, or other ligand receptors that are fragments of antibodies, and that retain the binding specificity of the antibody (e.g., an Fab fragment, Fab' fragment, etc.)." Appeal Brief, page 26.

In response to this argument, the examiner states "it is not clear what are the standard immunological techniques used to prepare the ligand receptor." Examiner's Answer, page 18. From the statement of the rejection and the response to appellants' arguments it is apparent that the examiner has not used the correct legal standard in making the rejection. "[T]he definiteness of the language employed [in a claim] must be analyzed--not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary level of skill in the pertinent art." In re Moore, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (CCPA 1971)(footnote omitted). Here the examiner has considered the referenced term in a vacuum. There is no analysis of the prior art or the application disclosure. On this record, the examiner has not established a prima facie case of indefiniteness.

The examiner next questions the language found in claims 98-99 "in an amount sufficient to inhibit binding of the linkage siteof at least one ligand conjugate to an

antibody or receptor" The examiner states that it is "not clear which amount would be sufficient to inhibit binding of the linkage site to the receptor. The specification nowhere teaches the amount necessary for the inhibition of binding of the linkage site. Applicants are requested to include the amount of inhibitors required." Examiner's Answer, page 10.

The examiner's request for appellants to "include the amount of inhibitors" in the claims is unreasonable. As explained in In re Mattison, 509 F.2d 563, 184 USPQ 484 (CCPA 1975), it is proper for a patent applicant to define their invention through use of functional amounts provided the specification sets forth sufficient guidance so that one skilled in the art would be able to determine an appropriate amount. Here, the examiner has focused on the lack of numerical limitations in the claims and has not analyzed the specification of this application and explained why there is insufficient guidance that would allow one to arrive at an appropriate amount.

Finally, the examiner rejects claim 102 for reciting "drug of abuse, metabolite of drug abuse, and analogue of the drug abuse . . . and an analogue of a metabolite of a therapeutic drug." The examiner merely states that "the specification does not recite that the ligand can be either of those. The specification defines the ligand is a binding partner of a receptor." As set forth above in regard to the examiner's rejection of claim 102 under 35 U.S.C. § 112, first paragraph (written description), we find that the specification of this application does describe these compounds as being the ligand of interest in the present invention.

The examiner's rejection under 35 U.S.C. § 112, second paragraph, is reversed.

5. Rejection of Claims 98-102 as Anticipated by Kinoshita.

The examiner rejects these claims stating "Kinoshita et al teach immunoassay for captopril comprising a plurality of molecules of captopril-MCC-beta-galactosidase (refers to the ligand analogue conjugate of the instant claims) and maercaptoethanol[sic]-MCC (refers to crosstalk inhibitors of the instant claims). Thus, the reference clearly anticipates the claimed invention." Examiner's Answer, page 10.

With regard to claim 98, appellants point out that this claim requires that the composition comprise a "plurality of different ligand analogue conjugates." Appeal Brief, page 13. The examiner has not pointed out where Kinoshita describes a composition which comprises a plurality of different ligand analogue conjugates. Thus, we reverse the rejection as it pertains to claim 98.

We also reverse the rejection as it pertains to claims 99-102. Claims 99-102 require a composition which comprises a defined ligand analogue conjugate, ligand receptor, and crosstalk inhibitor. The examiner's statement of the rejection takes into account only the ligand analogue conjugate and crosstalk inhibitor. Nowhere does the statement of the rejection take into account the ligand receptor. Thus, even assuming arguendo the examiner correctly correlated the stated compounds of Kinoshita to the ligand analogue conjugate and crosstalk inhibitor in claims 99-102, the statement of the rejection does not take into account the subject matter of any claim as a whole.

Furthermore, the examiner has merely pointed to two compounds described in the reference. The claimed invention is directed to a composition which contains three specified compounds. The examiner has not pointed to any composition described in Kinoshita which comprises compounds which meet the requirements of the claims 99-102.

The examiner's rejection of claims 98-102 under 35 U.S.C. § 102(b) based upon Kinoshita is reversed.

6. Rejection of claims 99-102 as anticipated by Marini.

The reasons given by the examiner in regard to this rejection are that:

Marini et al teach a simple procedure to bind to haptens, drugs, peptides (refers to ligand analogues of the instant claims) selectively through their amino or carboxyl group to a spacer (refers to linkage site of the instant claims). The reference specifically teach conjugation of chemical spacers to acetylated gelatin (refers to crosstalk inhibitor comprising analogue of the linkage site). The reference anticipates the claimed invention.

Examiner's Answer, page 11. Again, the examiner has not taken into account that claims 99-102 require three components. The examiner has not pointed to any specific composition described in Marini which comprises the three components required by claims 99-102 on appeal.

The examiner's rejection of claims 99-102 as anticipated by Marini is reversed.

7. Rejection of claims 99-102 as anticipated by Schuurs.

The examiner's statement of the rejection reads as follows: "Schuurs et al. teach immuno assay composition comprising, ligand analogue conjugate (estroidal-17-succinyl-HRP), a cross talk inhibitor (estroil which has analogue of the linkage site). The reference clearly anticipates the claimed invention." Examiner's Answer, page 11. Once again, the examiner has not taken into account that the compositions of claims 99-102 must have three components not two and has not pointed to any specific composition described in Schuurs which comprises the three components required by claims 99-102.

The examiner's rejection of claims 99-102 under 35 U.S.C. § 102(b) as anticipated by Schuurs is reversed.

The decision of the examiner is reversed.

REVERSED

)
William F. Smith)
Administrative Patent Judge)
)
)
)
) BOARD OF PATENT
Demetra J. Mills)
Administrative Patent Judge) APPEALS AND
)
) INTERFERENCES
Eric Grimes)
Administrative Patent Judge)

Foley & Lardner
402 West Broadway
23rd Floor
San Diego, CA 92101

dem

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte JAY K. BASS, JOHN F. McENTEE, TIM J. LAZARUK,
MARYAM MOBED-MIREMADI, and BRENT T. TOLOSKO

Appeal 2009-008347
Application 10/939,952
Technology Center 1600

Decided: February 16, 2010

Before DONALD E. ADAMS, DEMETRA J. MILLS, and
LORA M. GREEN, *Administrative Patent Judges*.

ADAMS, *Administrative Patent Judge*.

DECISION ON APPEAL

This appeal under 35 U.S.C. § 134 involves claims 1, 3-24, 26-35, and 37, the only claims pending in this application. We have jurisdiction under 35 U.S.C. § 6(b).

STATEMENT OF THE CASE

The claims are directed to an apparatus for preparing an array of chemical compounds/biopolymers on the surface of a support. Claims 1 and 4 are illustrative:

1. An apparatus for preparing an array of chemical compounds on the surface of a support, said apparatus comprising;

two elements that are disposed relative to one another to form a gap between said elements wherein the width of said gap is sufficient such that a flow of gas moving outwardly along a perimeter of said gap forms an aerodynamic seal between said two elements and such that said two elements are in a movable relationship relative to one another during said aerodynamic seal and that form a chamber having a controllable interior environment for preparing said array of chemical compounds and

a mechanism for introducing a gas into said gap.

4. An apparatus according to Claim 1 wherein one of said two elements comprises a bottom wall and side walls wherein said mechanism for introducing a gas comprises (i) openings along an upper surface of said side walls and (ii) a source of said gas.

The Examiner relies on the following evidence:

Brennan	US 6,001,311	Dec. 14, 1999
Cerrina et al.	US 6,375,903 B1	Apr. 23, 2002

The rejections presented by the Examiner follow:

1. Claims 1 and 3-20 stand rejected under the written description provision of 35 U.S.C. § 112, first paragraph.

2. Claims 1, 3, 5-7, 9-15, 17-22, 24, 27-33, 35, and 37 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Brennan.
3. Claims 4, 6, 23, and 24 stand rejected under 35 U.S.C. § 103(a) as unpatentable over the combination of Brennan and Cerrina.

We reverse.

Written Description:

ISSUE

Have Appellants established error in the Examiner's conclusion that Appellants' Specification fails to disclose a gap width sufficient to form an aerodynamic seal?

FINDINGS OF FACT

FF 1. The Examiner finds that "page 8, lines 6-8 and 31-33" of Appellants' Specification fails to "define or describe a gap width sufficient to form an aerodynamic seal as newly claimed" (Ans. 3).

FF 2. The Examiner finds that while "page 11, lines 11-16; page 13, lines 20-29; page 18, lines 1-5; and Fig. 1 . . . describe an aerodynamic seal within the gap and illustrate a gap" since Appellants' "specification does not define what is encompassed by the gap width 'sufficient' for forming the seal" (Ans. 10).

FF 3. The Examiner finds that Appellants' Specification "use[s] the phrase 'size of the gap', however the specification does not teach dimensions (e.g. width) encompassed by the 'size' . . . [or] a positional relationship defined by the 'width'" (*id.*).

FF 4. Appellants' Specification discloses that

An aerodynamic seal between the top element and the bottom element is realized by introducing a gas into the gap between the top element and the bottom element. The pressure of the gas is dependent on a number of factors including the size of the gap, the pressure of any gas introduced into the interior of the chamber formed by the top element and the bottom element, the velocity of the stages, and so forth.

(Spec. 11: 12-16.)

FF 5. Appellants' Specification discloses that "[i]n some embodiments there is a pressure differential from the middle to the edges (both inside and outside) of the seal, established by the size of the gap, the flow through it and the width and profile of the sealing surface. . . . [T]he magnitude of this differential protects against recirculation and entrainment of atmospheric air by having the remaining seal flow predictably exit the seal gap" (Spec. 13: 20-28).

PRINCIPLES OF LAW

The Examiner has the initial burden of presenting evidence or reasoning to explain why persons skilled in the art would not recognize in the original disclosure a description of the invention defined by the claims.

In re Wertheim, 541 F.2d 257, 263 (CCPA 1976).

"In order to satisfy the written description requirement, the disclosure as originally filed does not have to provide *in haec verba* support for the claimed subject matter at issue." *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323 (Fed. Cir. 2000). A disclosure provides adequate written description if it conveys with reasonable clarity to those skilled in the art that the inventor was in possession of the invention. *See id.*

ANALYSIS

The apparatus of Appellants' claim 1 comprises, *inter alia*, two elements separated by a gap that has a width sufficient to allow a flow of gas moving outwardly along a perimeter of the gap to form an aerodynamic seal between the elements. In contrast, Appellants' claim 21 requires two (top and bottom) elements that are separated by a gap, wherein an aerodynamic seal between the two elements comprises a flow of gas outwardly along a perimeter of said gap.

While the Examiner does not dispute that Appellants' Specification has written descriptive support for Appellants' claim 21, the Examiner asserts that Appellants' Specification "provides no description of a gap width 'sufficient' for forming an aerodynamic seal. Hence, one of skill in the art would not understand that Appellant had possession of the claimed invention at the time of filing" (Ans. 11). In this regard, the Examiner inquires as to whether the term "'width' define[s] the distance between the top element . . . and bottom element . . . or . . . the distance between side walls" (Ans. 10-11). We are not persuaded by the Examiner's inconsistent position.

While claim 21 does not include a phrase defining the width of the gap as sufficient to form an aerodynamic seal, there is no doubt that the gap in claim 21 has a width and that this width is sufficient to form an aerodynamic seal as required by the claim. As Appellants' Specification discloses an aerodynamic seal between two elements is realized by introducing a gas into the gap between the two elements and that the pressure of the gas introduced into the gap is dependent upon, *inter alia*, the size of the gap (FF 4). Accordingly, if there is adequate written descriptive

support in Appellants' Specification for claim 21 then, absent persuasive reasoning and/or evidence from the Examiner, this same disclosure would cut against the Examiner's concern regarding claims 1 and 3-20.

In sum, we find that the Examiner failed to provide a sufficient evidentiary basis on this record to support his conclusion that Appellants' Specification fails to provide written descriptive support for the phrase "wherein the width of said gap is sufficient such that a flow of gas moving outwardly along a perimeter of said gap forms an aerodynamic seal between said two elements" (Ans. 3). Accordingly, we agree with Appellants' contention that a person of ordinary skill in this art reading Appellants' Specification (*see e.g.*, FF 4 and 5) "would not doubt that the Appellants had possession of the claimed invention" (App. Br. 13).

CONCLUSION OF LAW

Appellants established error in the Examiner's conclusion that Appellants' Specification fails to disclose a gap width sufficient to form an aerodynamic seal.

We reverse the rejection of claims 1 and 3-20 under the written description provision of 35 U.S.C. § 112, first paragraph.

Anticipation:

ISSUE

Have Appellants established that Brennan fails to teach an apparatus wherein an aerodynamic seal is formed by the flow of gas moving outwardly along a perimeter of a gap between two elements as required by Appellants' claimed invention?

FINDINGS OF FACT

FF 6. For clarity we reproduce Brennan's Figure 3 below:

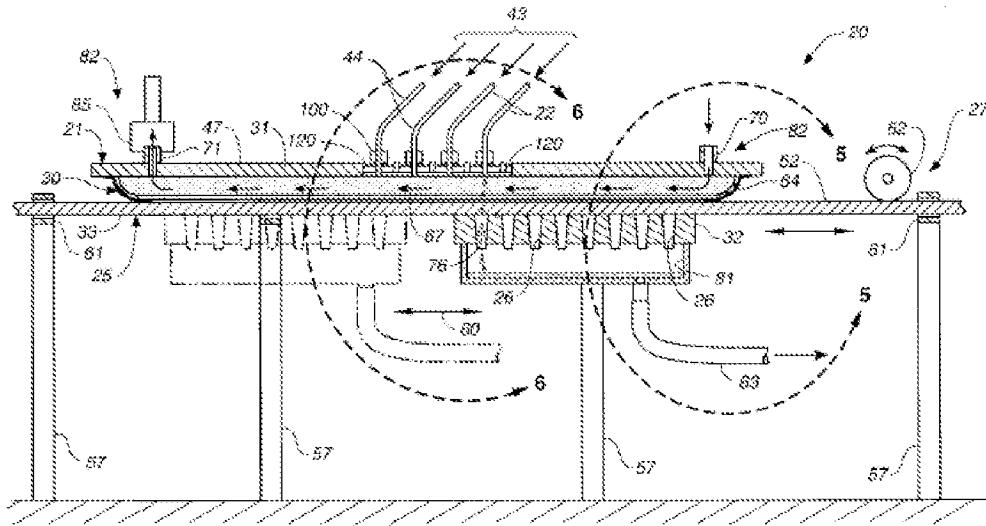


FIG. 3

"FIG. 3 is a side elevation view, in cross-section of the chemical synthesis array apparatus of . . . [Brennan's invention] showing the sweeping action of the flow of inert gas through the common chamber and showing a cross-sectional view through the sliders and sliding seals of the nozzle columns" (Brennan, col. 5, ll. 42-46).

FF 7. The Examiner finds that a gap exists between head assembly (21) and base (25) of Brennan's apparatus and that these two elements are in a

sealed, movable relationship that maintains an aerodynamic seal within the gap (Ans. 3).

FF 8. The Examiner finds that “[t]he aerodynamic seal is evidenced by the arrows illustrating gas flow going from left to right in Fig. 3, but not going down through the reaction sites (#76)” and “is provided by maintaining a positive pressure within the chamber to control flow through the chamber, wherein the chamber is relatively small such that the gas flow can ‘sweep or flush’ the chamber” (Ans. 4).

FF 9. The Examiner finds that the gas flow in Brennan’s apparatus “sweeps the perimeter because the perimeter is part of the chamber which is swept. This is evidenced by the fact that if a leak occurs, it escapes along the edge” (Ans. 12).

FF 10. Brennan teaches that “it is important to normally maintain a minimum positive pressure inside common chamber 31 at all times during synthesis which is slightly greater than atmospheric pressure so that the flow of gas, should a leak occur, would be outward” (Brennan, col. 14, ll. 22-26).

PRINCIPLES OF LAW

“[T]he [E]xaminer bears the initial burden, on review of the prior art or on any other ground, of presenting a *prima facie* case of unpatentability.” *In re Oetiker*, 977 F.2d 1443, 1445 (Fed. Cir. 1992). On appeal to this Board, Appellants must show that the Examiner has not sustained the required burden. *See Ex parte Yamaguchi*, 88 USPQ2d 1606, 1608 and 1614 (BPAI 2008) (precedential).

“A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior

art reference.” *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987).

ANALYSIS

The Examiner interprets Appellants’ claims as encompassing the “flow of gas so as to flush or sweep the chamber as taught by Brennan” (Ans. 4; FF 8-9). In this regard, the Examiner reasons that Appellants’ claims do

[N]ot limit gas flow such that the [sic] flows only to the perimeter, but encompasses the entire chamber which includes the perimeter. . . . Gas flow sweeping the chamber, also sweeps the perimeter because the perimeter is part of the chamber which is swept. This is evidenced by the fact that if a leak occurs, it escapes along the edge. If the sweeping did not also sweep the perimeter, no gas would reach the edge and hence could not possibly leak.

(Ans. 12; FF 9.) We are not persuaded.

Appellants’ claims require a flow of gas outwardly along a perimeter of a gap. In Brennan the gas flows *along* a perimeter of a gap, not outwardly *along* the perimeter (FF 6-8). As the Examiner realizes, the gas in Brennan’s apparatus flows outwardly only if a leak develops in the perimeter (FF 9-10). Accordingly, we agree with Appellants’ contention that in Brennan’s apparatus, an outward flow of gas only occurs if a “leak” occurs and then the “outward flow of gas from a leak . . . would occur at one point (i.e., the leak site) on the perimeter and not along the ‘length of such a boundary’” (App. Br. 15).

CONCLUSION OF LAW

Appellants established that Brennan fails to teach an apparatus wherein an aerodynamic seal is formed by the flow of gas moving outwardly along a perimeter of a gap between two elements as required by Appellants' claimed invention.

The rejection of claims 1, 3, 5-7, 9-15, 17-22, 24, 27-33, 35, and 37 under 35 U.S.C. § 102(b) as being anticipated by Brennan is reversed.

Obviousness:

ISSUE

Have Appellants established that the combination of Brennan and Cerrina fails to teach an apparatus wherein an aerodynamic seal is formed by the flow of gas moving outwardly along a perimeter of a gap between two elements as required by Appellants' claimed invention?

FINDINGS OF FACT

- FF 11. The Examiner relies on Brennan as discussed above (*see* Ans. 9).
- FF 12. The Examiner finds that Brennan fails to teach "the top or bottom element comprises glass" (*id.*).
- FF 13. The Examiner relies on Cerrina to "teach a device . . . comprising two elements disposed relative to each other to form a space between wherein . . . one of the elements comprises glass" (*id.*).

PRINCIPLES OF LAW

"[T]he [E]xaminer bears the initial burden, on review of the prior art or on any other ground, of presenting a *prima facie* case of unpatentability."

In re Oetiker, 977 F.2d 1443, 1445 (Fed. Cir. 1992). On appeal to this Board, Appellants must show that the Examiner has not sustained the required burden. *See Ex parte Yamaguchi*, 88 USPQ2d 1606, 1608 and 1614 (BPAI 2008) (precedential); *Ex parte Fu*, 89 USPQ2d 1115, 1118 and 1123 (BPAI 2008) (precedential).

“The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.” *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 416 (2007). It is proper to “take account of the inferences and creative steps that a person of ordinary skill in the art would employ.” *Id.* at 418. *See also id.* at 421 (“A person of ordinary skill is also a person of ordinary creativity, not an automaton.”). In sum, the “suggestion test is in actuality quite flexible and not only permits, but *requires*, consideration of common knowledge and common sense.” *DyStar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick Co.*, 464 F.3d 1356, 1367 (Fed. Cir. 2006).

Nevertheless, an invention “composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art. . . . [I]t can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does.” *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007).

ANALYSIS

Appellants contend that Cerrina fails to make up for the deficiency in Brennan discussed above (App. Br. 26). In response, the Examiner asserts

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Application 10/939,952

that "Brennan is not deficient in teaching this element" of the claims" (Ans. 18). We disagree for the reasons set forth above.

CONCLUSION OF LAW

Appellants established that the combination of Brennan and Cerrina fails to teach an apparatus wherein an aerodynamic seal is formed by the flow of gas moving outwardly along a perimeter of a gap between two elements as required by Appellants' claimed invention.

The rejection of claims 4, 6, 23, and 24 under 35 U.S.C § 103(a) as unpatentable over the combination of Brennan and Cerrina is reversed.

REVERSED

alw

AGILENT TECHNOLOGIES, INC.
LEGAL DEPARTMENT, DL429
INTELLECTUAL PROPERTY ADMINISTRATION
P.O. BOX 7599
LOVELAND, CO 80537-0599



US06432050B1

(2) United States Patent
Porat et al.

(10) Patent No.: US 6,432,050 B1
(11) Date of Patent: Aug. 13, 2002

(54) IMPLANTABLE ACOUSTIC BIO-SENSING
SYSTEM AND METHOD

(75) Inventors: Yariv Porat, Haifa; Avi Penner, Tel Aviv; Eyal Doron, Kiryat Yam, all of (IL)

(73) Assignee: Remon Medical Technologies Ltd., Caesarea (IL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/303,644

(22) Filed: May 3, 1999

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/161,656, filed on Sep. 29, 1998, now Pat. No. 6,237,398, which is a continuation-in-part of application No. 09/780,553, filed on Dec. 30, 1997, now Pat. No. 6,140,740.

(51) Int. Cl. 7 A61B 5/00

(52) U.S. Cl. 600/300, 600/309, 600/500, 128/899

(58) Field of Search 600/300, 309, 600/310, 323, 339, 500, 502, 504, 505, 128/899, 903, 607/30, 31, 32, 33, 60, 61

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Primary Examiner—Eric F. Winakur

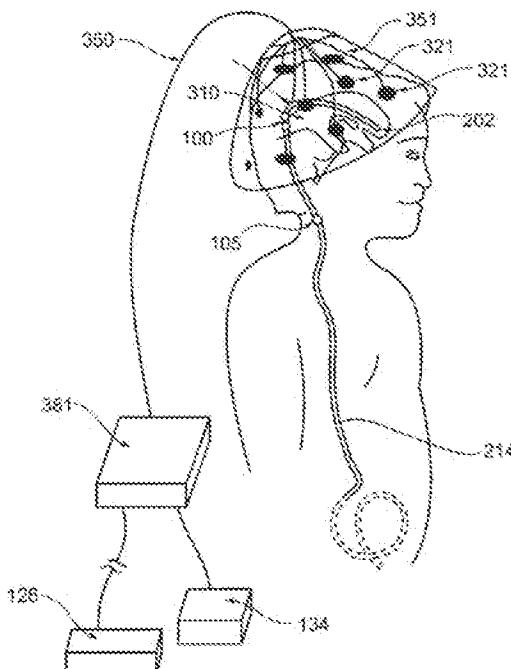
Assistant Examiner—Joseph A. Cadigan

(74) Attorney, Agent, or Firm—G. E. Erdlich Ltd.

(57) ABSTRACT

An implantable biosensor system for monitoring and optionally alleviating a physiological condition in a patient is provided and includes (a) at least one sensor for sensing at least one parameter of a physiological condition and for generating electrical sensor signals representative of the physiological condition; and (b) a first acoustic activatable transducer being directly or indirectly coupled with the at least one sensor, the first acoustic activatable transducer being for converting a received acoustic interrogation signal from outside the patient's body into an electrical power for energizing the processor, the first acoustic activatable transducer further being for converting the electrical sensor signals of the at least one sensor into acoustic signals receivable out of the patient's body, such that information pertaining to the at least one parameter of the physiological condition can be relayed outside the patient's body upon generation of an acoustic interrogation signal.

3 Claims, 11 Drawing Sheets



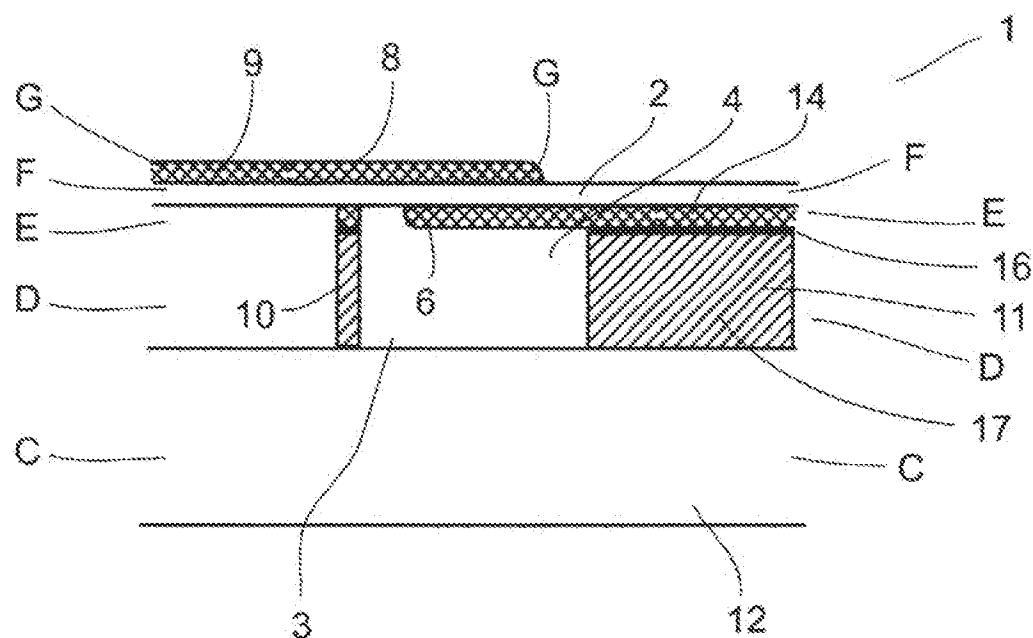


Fig. 1a

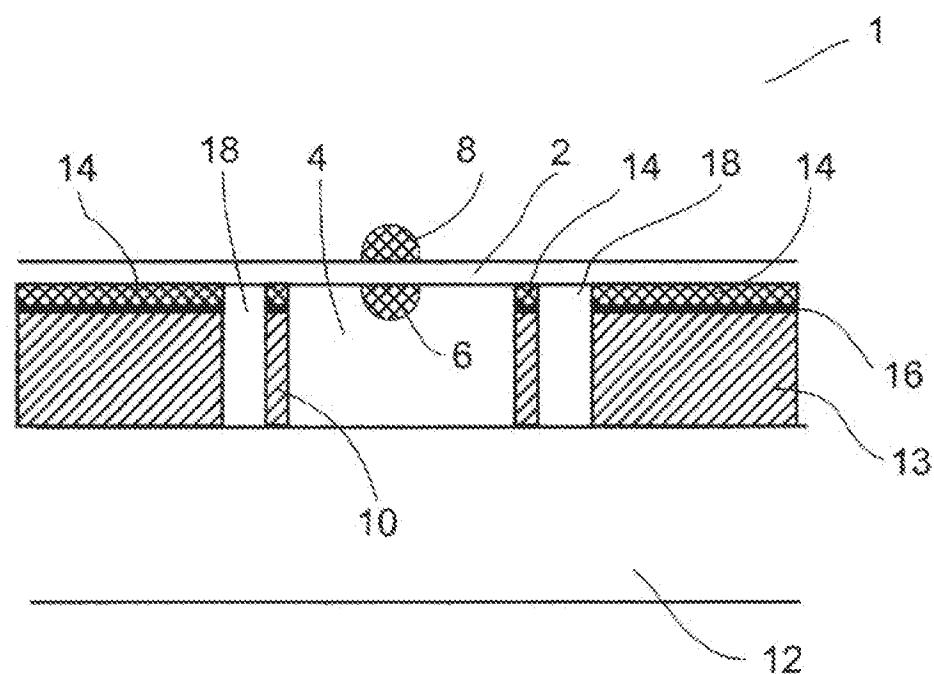


Fig. 1b

Fig. 2a

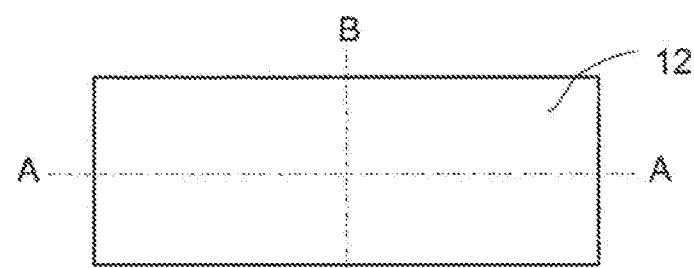


Fig. 2b

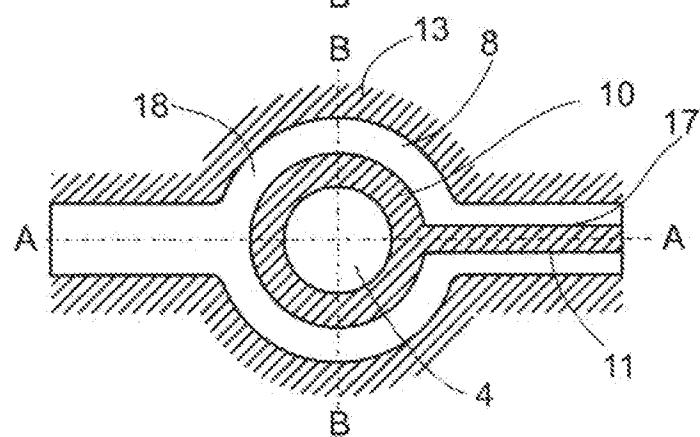


Fig. 2c

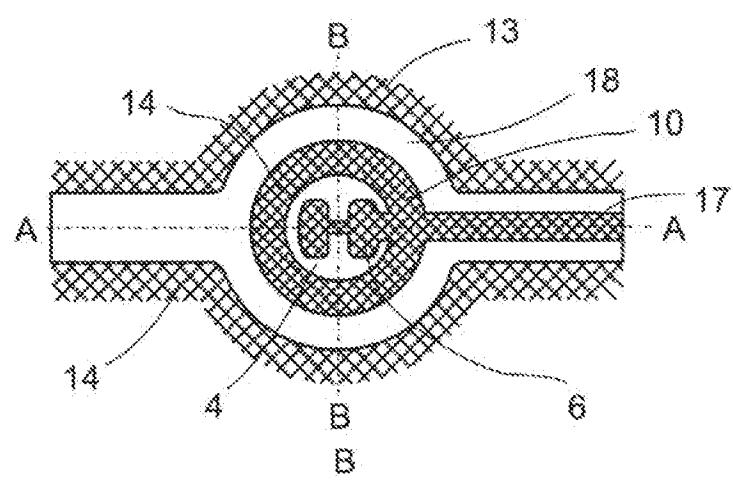


Fig. 2d

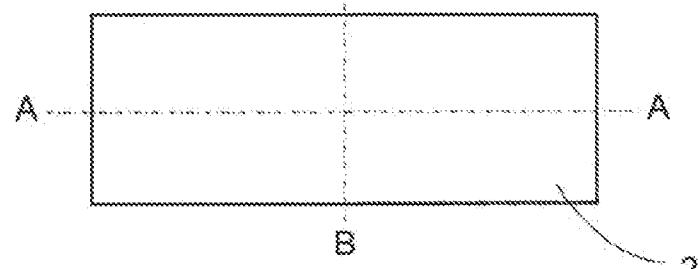


Fig. 2e

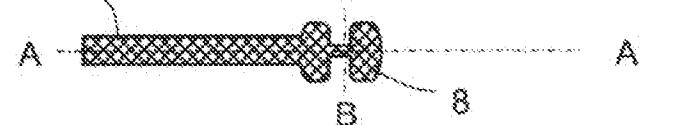


Fig. 3

Transverse direction

Charge contribution on a PVDF disk

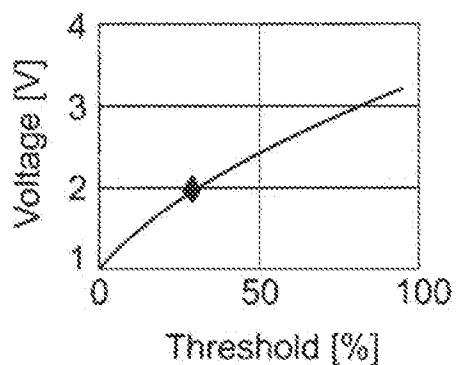
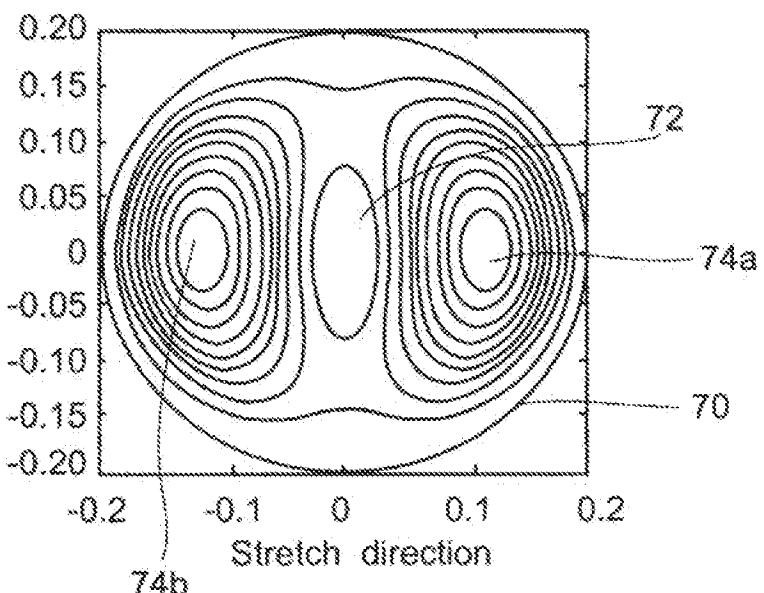


Fig. 4a

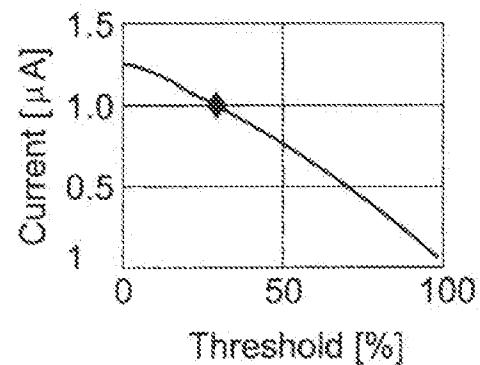


Fig. 4c

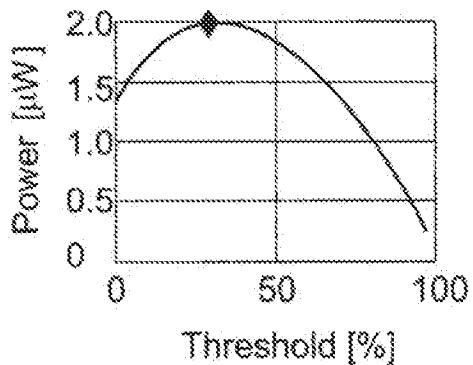


Fig. 4b

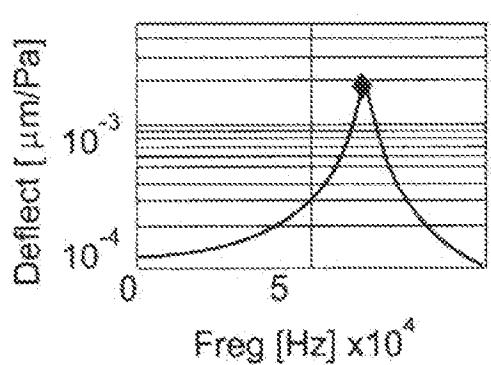


Fig. 4d

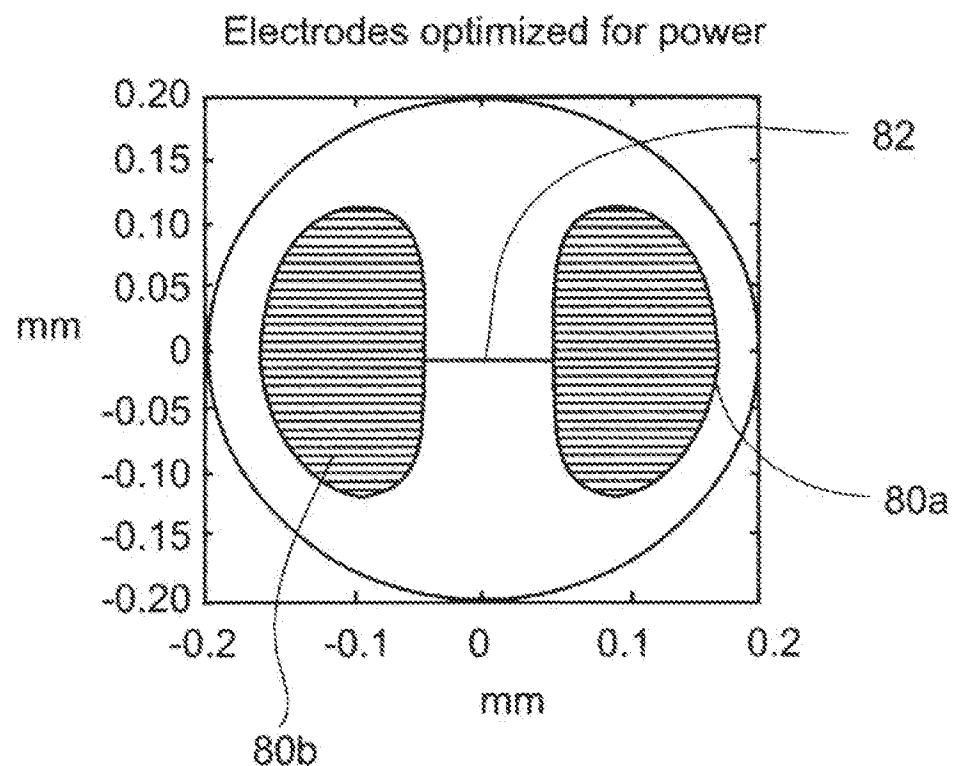


Fig. 5

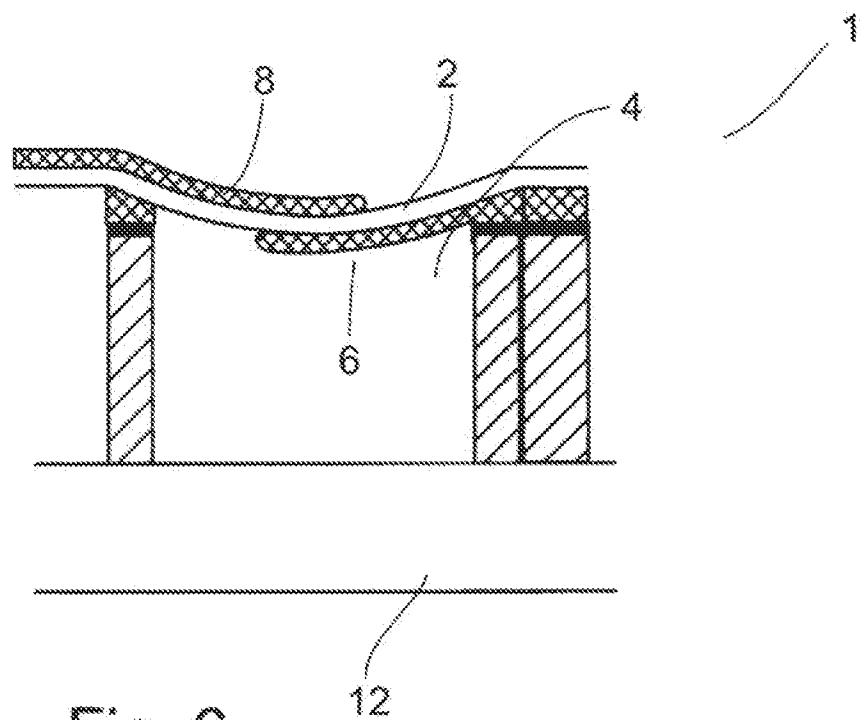


Fig. 6

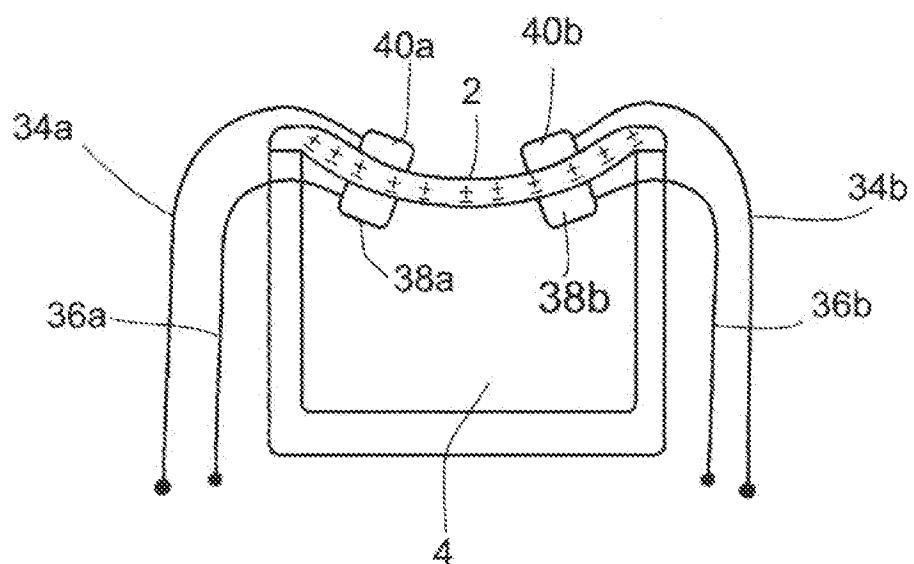


Fig. 7a

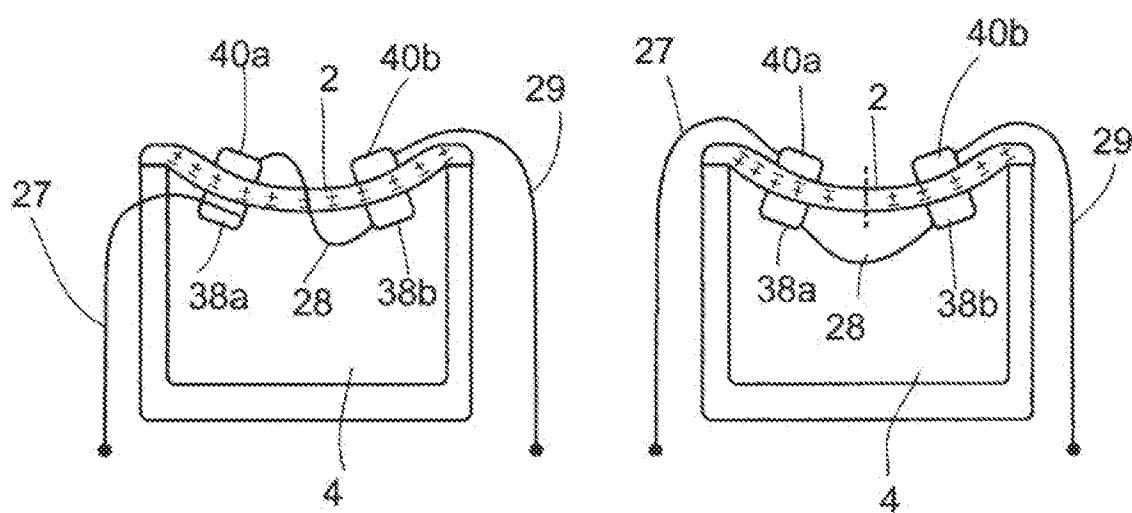


Fig. 7b

Fig. 7c

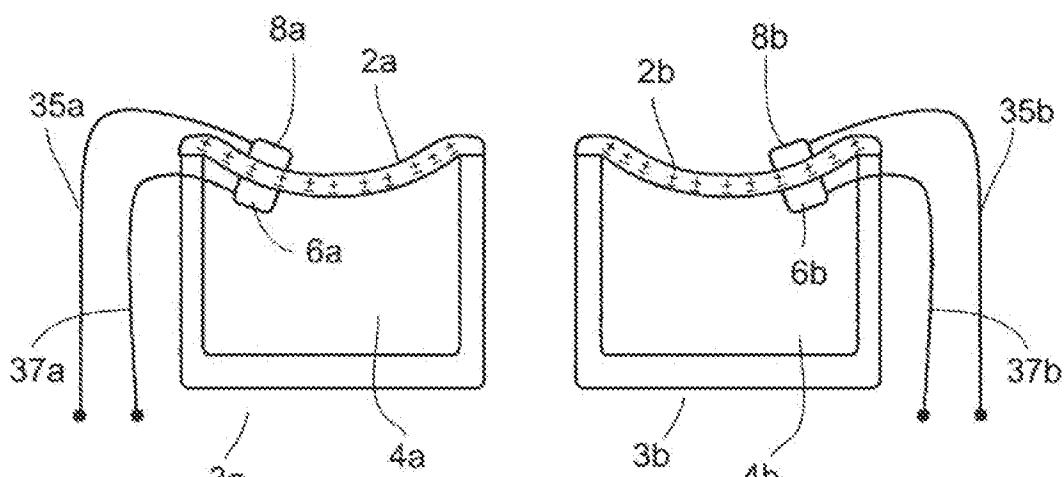


Fig. 7d

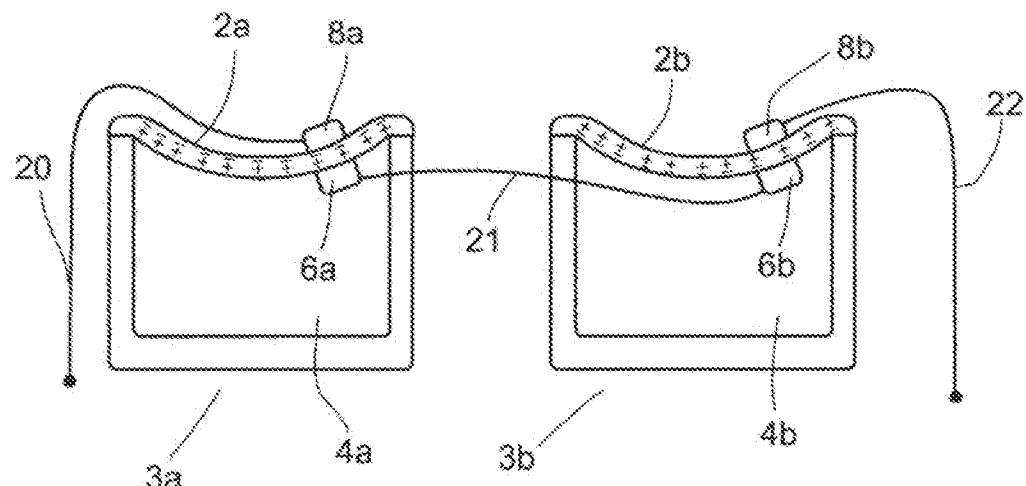


Fig. 7e

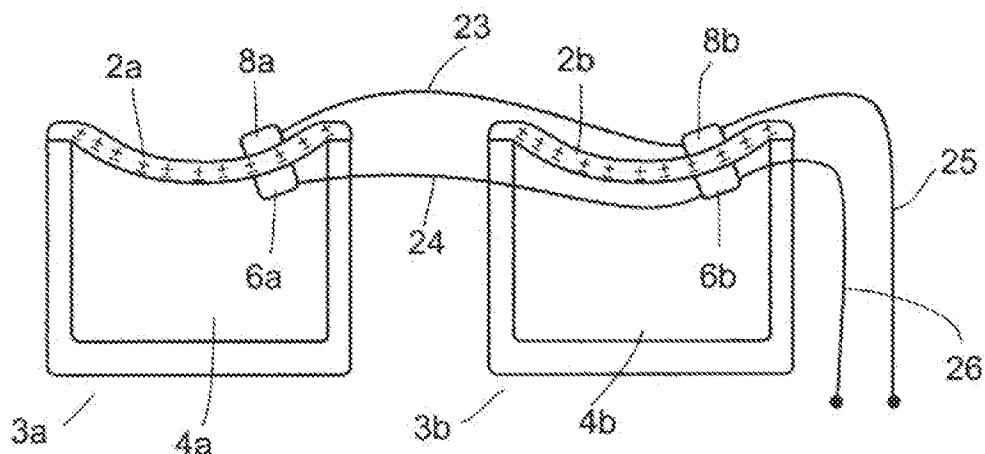


Fig. 7f

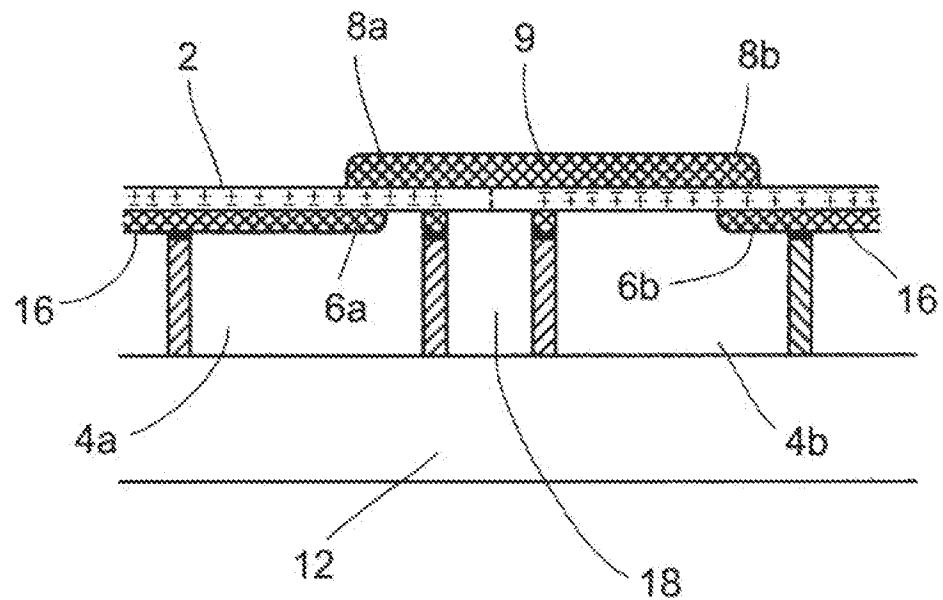


Fig. 8

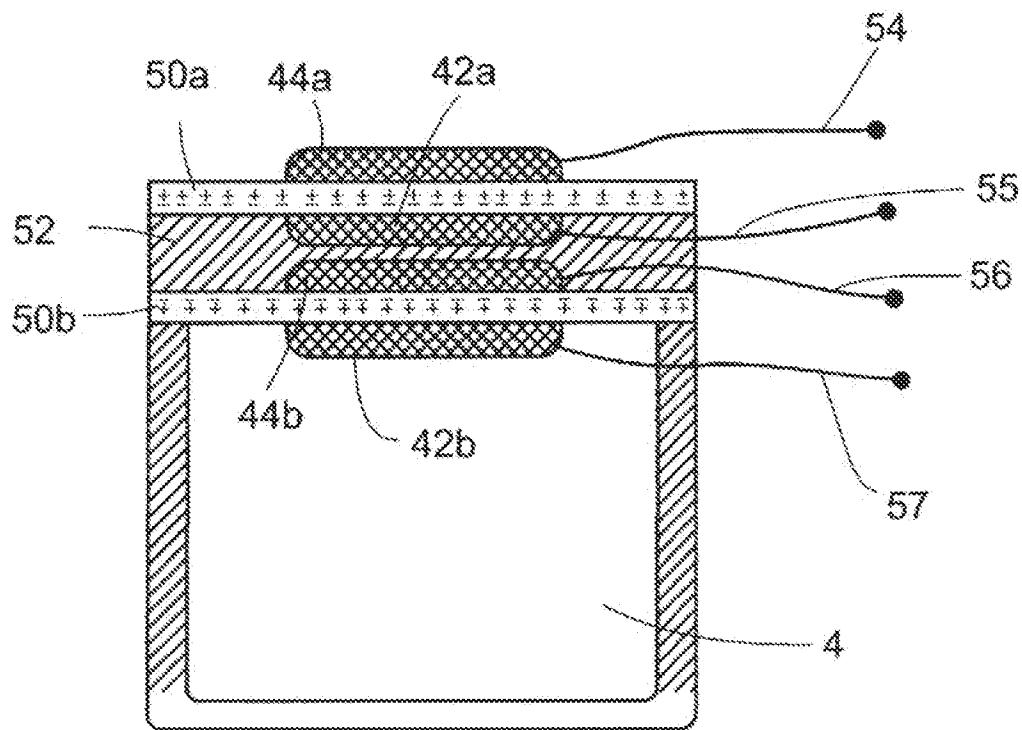


Fig. 9

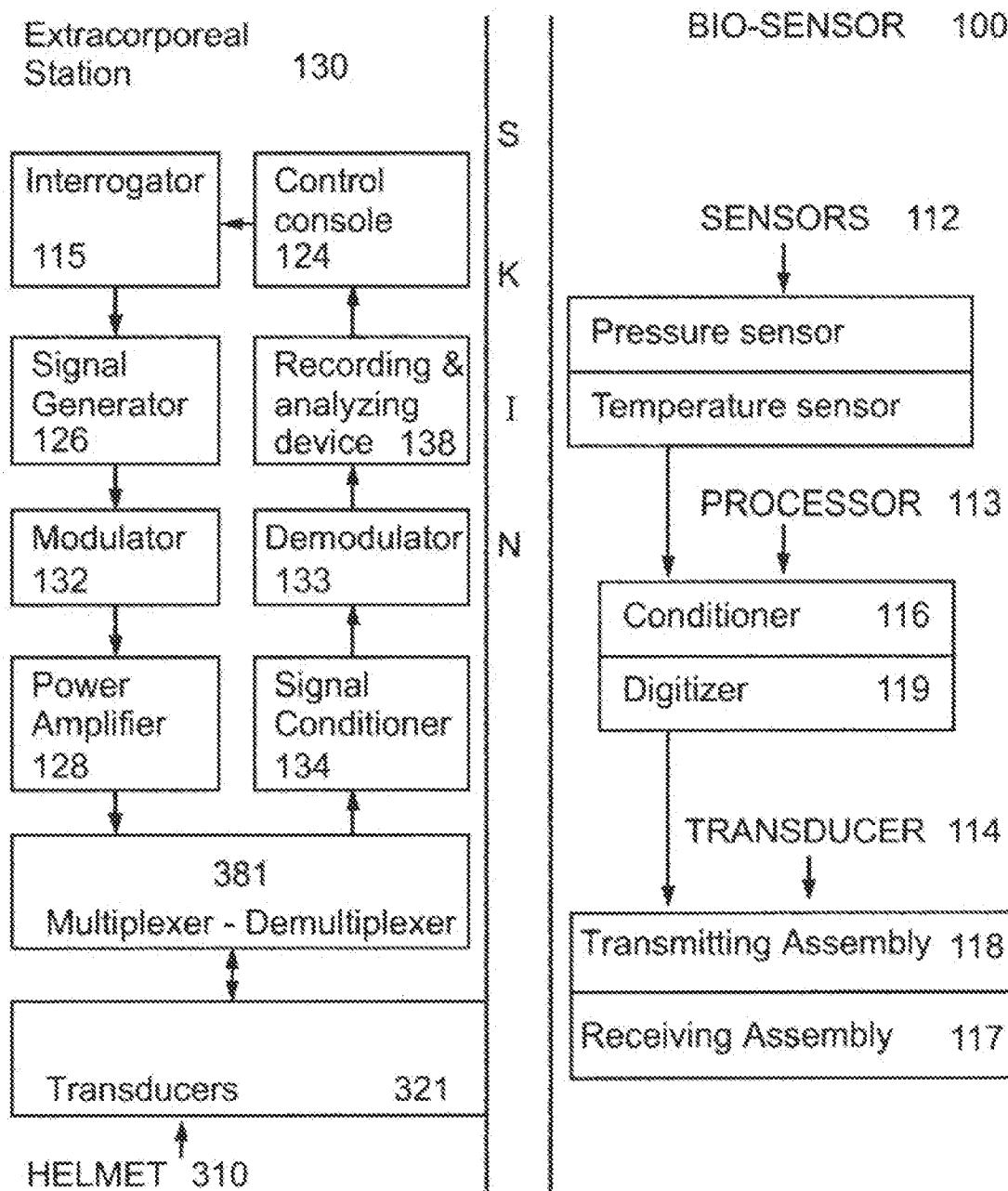


Fig. 10

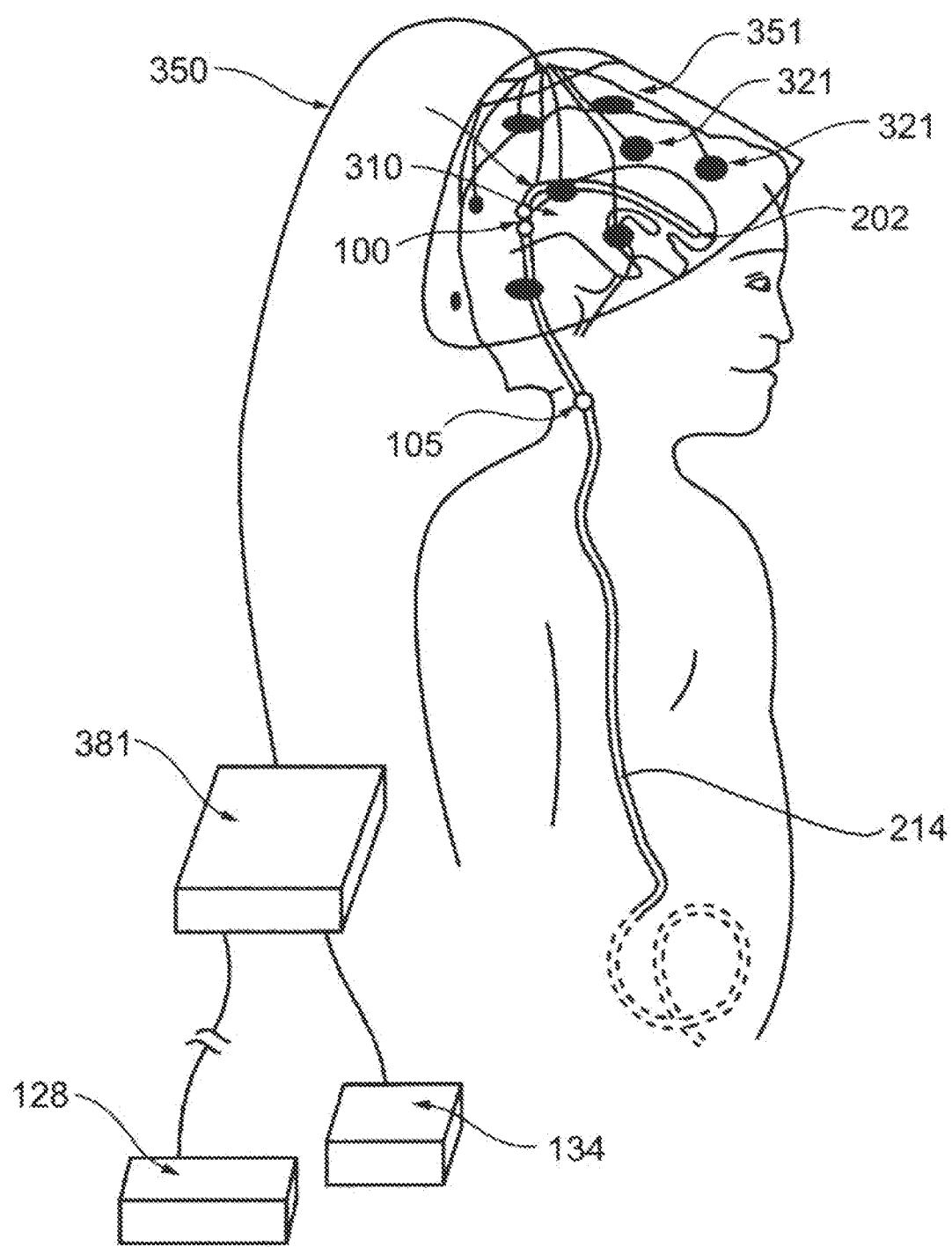


Fig. 11

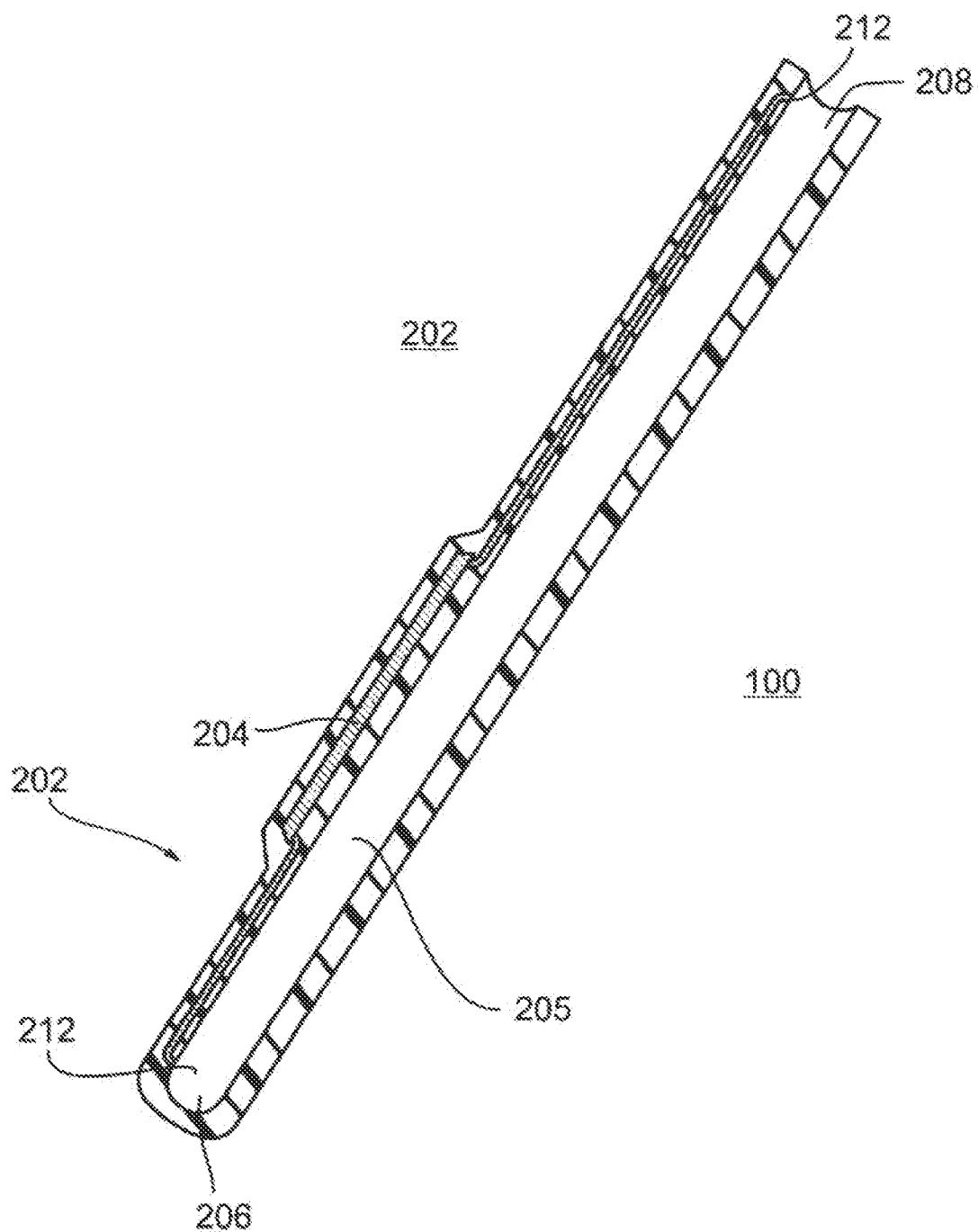


Fig. 12

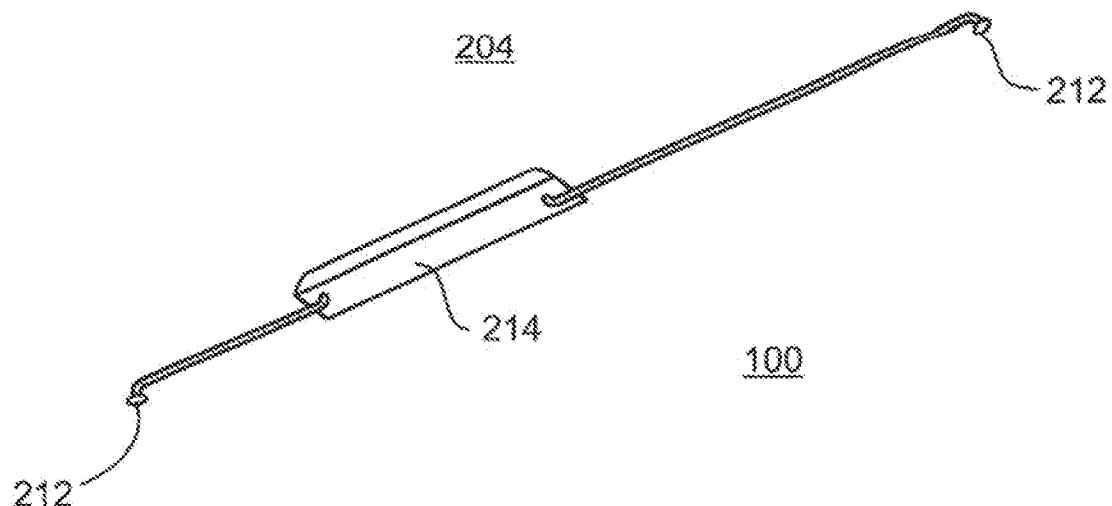


Fig. 13

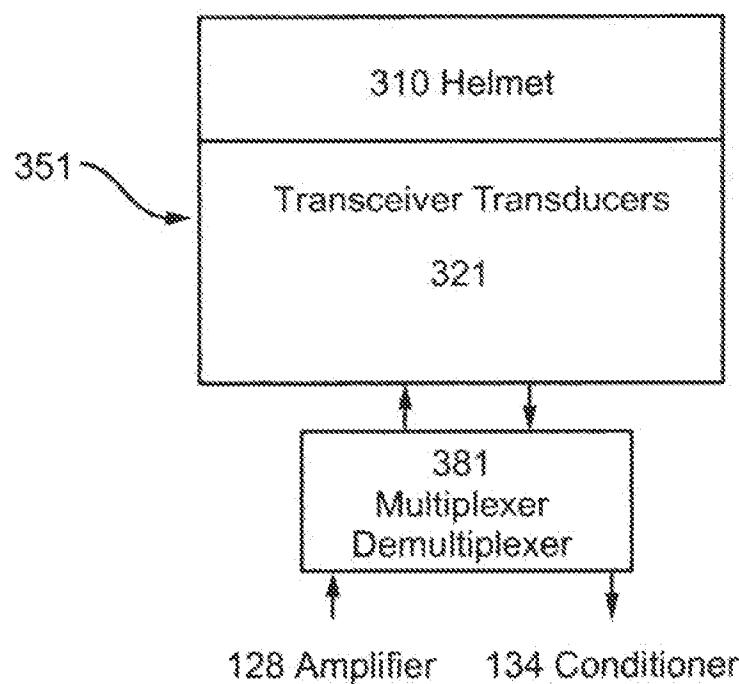


Fig. 14

1
**IMPLANTABLE ACOUSTIC BIO-SENSING
SYSTEM AND METHOD**

This is a continuation-in-part of U.S. patent application Ser. No. 09/161,658, filed Sep. 29, 1998, now U.S. Pat. No. 6,237,398, issued May 29, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 09/000,553, filed Dec. 30, 1997, now U.S. Pat. No. 6,140,740, issued Oct. 31, 2000.

FIELD AND BACKGROUND OF THE
INVENTION

The present invention relates to a biosensing system and method for monitoring internal physiological conditions of a patient. More particularly, the present invention relates to a biosensor system implantable in a patient's body that includes at least one sensor, an active acoustic transducer and a miniature processor. The sensor is used to monitor a physiological condition of the patient and relay information pertaining to the physiological condition through the miniature processor to the active acoustic transducer. The active acoustic transducer transmits this information out of the patient's body as an acoustic signal. Transmission of an acoustic signal from the transducer is triggered by an externally generated acoustic interrogation and energizing signal, which is produced by a second acoustic transducer positioned externally, yet in intimate contact with, the patient's body. The miniature electronic processor is utilized for the various required functions such as conditioning, digitization and amplification of the sensor signals. The biosensor of the present invention can also include a shunt and a monitoring device embedded in the walls of the shunt for permitting identification and non-invasive testing of the operation of the shunt via the acoustic transducer.

Many medical conditions require the monitoring and measurement of internal physiological conditions of a patient. For example, hydrocephalus, which is a brain condition where cerebrospinal fluid accumulates at abnormally high pressures in ventricles or chambers of a patient's brain, may require monitoring of the intra-cranial fluid pressure of the patient.

Implantable devices for monitoring internal physiological conditions of a patient are known in the art. One such prior art device includes an implantable pressure sensor that transmits pressure signals out of the patient by mechanism of a wire or contact passing through the patient's skull (see, for example, U.S. Pat. No. 4,677,985). These types of devices are generally unsatisfactory due to increased risk of infection and patient discomfort caused by the externally extending wire.

Monitoring devices that are completely implantable within a patient are also known in the art. One such prior art device is described in U.S. Pat. No. 4,471,786 and includes a sensor for sensing a physiological condition of the patient and a transmitter and battery assembly for transmitting the sensor signals out of the patient's body. These types of devices are also unsatisfactory for many types of medical conditions since the batteries are bulky and must be periodically replaced, thus necessitating additional surgery.

Implantable monitoring devices that do not require batteries have also been developed. Such devices (see, for example, U.S. Pat. Nos. 3,943,915 and 4,593,703) employ sensors coupled with frequency tuned Lumped-Constant (L-C) circuits. The sensors mechanically translate changes in sensed physiological condition to the inductor or capacitor of the tuned L-C circuit for changing the reactance of the

L-C circuit. This change in reactance alters the resonant frequency of the circuit, which is then detected by an external receiver and converted to a signal representative of the monitored physiological condition.

Although these L-C type implantable monitoring devices are superior to battery operated devices in some respects, they also suffer from several limitations that limit their utility. For example, the L-C circuits are difficult to calibrate once implanted, are inherently single-channel, and are only sensitive in a particular range of measurements. Thus, L-C type monitoring devices are not always accurate after they have been implanted for a long period of time and are not suitable for use with sensors that have a wide sensing range. In addition, no processing power is provided.

Another implantable monitoring device that does not utilize wire connection or a battery supply makes use of large electromagnetic antennae to provide the energy required for the data processing inside the body. These antennae are big and risky to implant. Also, due to the high absorption of electromagnetic energy by human tissue, only subcutaneous implants are used, and energy into the depth of the body is realized by wiring coupling. Only small amounts of electromagnetic energy can be transmitted from an external antenna directly to a monitoring device deep in the body.

A general limitation of all of the above-described prior art implantable monitoring devices is that they are operable for sensing or monitoring only one physiological condition. Thus, if a doctor wishes to monitor, e.g., both the pressure and the temperature of the fluid in the ventricles of a patient's brain, two such devices must be implanted.

Furthermore, these prior art implantable devices merely monitor a physiological condition of the patient and transmit a signal representative of the condition out of the patient's body, but do not perform any processing or conversion of the signals.

In addition, due to inherent design limitations, these devices cannot be utilized for alleviating the underlying cause of the physiological condition monitored. For example, intra-cranial pressure sensors designed for use with patients suffering from hydrocephalus merely detect when fluid pressure levels within the patient's brain are high, but are not operable for reducing the amount of cerebrospinal fluid accumulated in the patient's brain. Thus, once these prior intra-cranial pressure sensors determine that the pressure in the patient's brain is too high, surgery must be performed to alleviate the condition.

An improved implantable biosensor for monitoring and alleviating internal physiological condition such as intracranial pressure has been described in U.S. Pat. No. 5,704,352 which discloses a biosensor system which includes at least one sensor for monitoring a physiological condition of the patient and a passive radio frequency transducer that receives sensor signals from the sensor or sensors, digitizes the sensor signals, and transmits the digitized signals out of the patient's body when subjected to an externally generated electromagnetically interrogation and energizing signal. The biosensor system described also includes a shunt, and as such it can be used for alleviating intracranial pressure monitored by the sensors of the biosensor.

Although this biosensor system presents a major advance over the above mentioned prior art devices and systems, it suffers from limitations inherent to the radio frequency transducer utilized thereby. Since this transducer requires the use of an antenna to receive and transmit signals, it possess limited reception and transmission capabilities due to the directional nature of such antennas. In addition, due to

the high absorption of electromagnetic energy by human tissue, deeply embedded implants cannot be realized by this system and as a result, the intra body positioning of such a biosensor is limited to regions close to the skin which are accessible to electromagnetic signals, thus greatly limiting the effectiveness of such a system.

There is thus a widely recognized need for, and it would be highly advantageous to have, a biosensor system for monitoring and alleviating internal physiological conditions, such as intra-cranial pressure, devoid of the above limitations.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a biosensor which can be used for non-invasive monitoring of body parameters.

It is another object of the present invention to provide such a biosensor which does not require wiring or an integral power source.

It is yet another object of the present invention to provide a biosensor which is less sensitive to extracorporeal positional effect when energized as compared to prior art devices.

It is still another object of the present invention to provide a biosensor which is effectively operable from any depth within the body.

To realize and reduce down to practice these objectives, the biosensor according to the present invention takes advantage of the reliable conductivity of acoustic radiation within water bodies, such as a human body and of an acoustic activatable piezoelectric transducer. According to one aspect of the present invention there is provided

According to one aspect of the present invention there is provided an implantable biosensor system for monitoring and optionally alleviating a physiological condition in a patient, the biosensor system comprising (a) at least one sensor for sensing at least one parameter of a physiological condition and for generating electrical sensor signals representative of the physiological condition; and (b) a first acoustic activatable transducer being directly or indirectly coupled with the at least one sensor, the first acoustic activatable transducer being for converting a received acoustic interrogation signal from outside the patient's body into an electrical power for energizing the processor, the first acoustic activatable transducer further being for converting the electrical sensor signals of the at least one sensor into acoustic signals receivable out of the patient's body, such that information pertaining to the at least one parameter of the physiological condition can be relayed outside the patient's body upon generation of an acoustic interrogation signal.

According to further features in preferred embodiments of the invention described below, the biosensor system further comprising a processor coupling between the at least one sensor and the first acoustic activatable transducer, the processor being for converting the electrical sensor signals into converted electrical signals representative of the physiological condition, the processor being energized via the electrical power.

According to another aspect of the present invention there is provided an implantable biosensor system for monitoring and alleviating a physiological condition in a patient, the biosensor system comprising (a) a shunt having a fluid passageway and being operable for draining fluid through the fluid passageway from a portion of a patient's body; (b)

a monitoring and operating mechanism coupled with the shunt for non-invasively monitoring the physiological condition and operating the shunt, the monitoring and operating mechanism including at least one sensor for sensing at least one parameter of the physiological condition and for generating electrical sensor signals representative of the physiological condition; and (c) a first acoustic activatable transducer being directly or indirectly coupled with the at least one sensor, the first acoustic activatable transducer being for converting a received acoustic interrogation signal from outside the patient's body into an electrical power for energizing the at least one sensor and for operating the shunt upon command, the first acoustic activatable transducer further being for converting the electrical sensor signals into acoustic signals receivable out of the patient's body, such that information pertaining to the at least one parameter of the physiological condition can be relayed outside the patient's body upon generation of an acoustic interrogation signal and the shunt is operable upon command.

According to still further features in the described preferred embodiments the monitoring and operating mechanism further includes a processor coupled with the at least one sensor, the processor serves for converting the electrical sensor signals to converted electrical signals representative of the physiological condition.

According to still further features in the described preferred embodiments the command is an acoustic operation signal provided from outside the body.

According to still further features in the described preferred embodiments the shunt is a cerebrospinal fluid shunt for draining cerebrospinal fluid from the patient's brain.

According to still further features in the described preferred embodiments the at least one sensor includes a first pressure sensor positioned within the fluid passageway for sensing the pressure of the cerebrospinal fluid in the patient's brain and for generating a first pressure signal representative of that pressure.

According to still further features in the described preferred embodiments the at least one pressure sensor includes a second pressure sensor positioned at a distance from the first pressure sensor and being for sensing the pressure of the cerebrospinal fluid when flowing through the shunt and for generating a second pressure signal representative of that pressure.

According to still further features in the described preferred embodiments the processor receives the first and second pressure signals from the first and second pressure sensors and calculates the flow rate of cerebrospinal fluid through the shunt.

According to still further features in the described preferred embodiments the first acoustic activatable transducer includes (i) a cell member having a cavity; (ii) a substantially flexible piezoelectric layer attached to the cell member, the piezoelectric layer having an external surface and an internal surface, the piezoelectric layer featuring such dimensions so as to enable fluctuations thereof at its resonance frequency upon impinging of the acoustic interrogation signal; and (iii) a first electrode attached to the external surface and a second electrode attached to the internal surface.

According to still further features in the described preferred embodiments the piezoelectric layer is of a material selected from the group consisting of PVDF and piezoceramic.

According to still further features in the described preferred embodiments the processor includes a conditioner and

a digitizer for converting the electrical sensor signal to the converted electrical signal.

According to still further features in the described preferred embodiments the converted electrical signal is a digital signal.

According to still further features in the described preferred embodiments the processor, the first acoustic activatable transducer and the at least one sensor are co-integrated into a single biosensor device.

According to still further features in the described preferred embodiments the biosensor system further comprising (c) an extracorporeal station positionable against the patient's body the extracorporeal station including an interrogation signal generator for generating the acoustic interrogation signal, the interrogation signal generator including at least one second transducer for transmitting the interrogation signal to the first acoustic activatable transducer and for receiving the receivable acoustic signals from the first acoustic activatable transducer.

According to still further features in the described preferred embodiments the processor includes a memory device for storing the electrical sensor signals and an analyzer for analyzing the electrical sensor signals.

According to still further features in the described preferred embodiments the processor includes a programmable microprocessor.

According to still further features in the described preferred embodiments the at least one sensor is selected from the group consisting of a pressure sensor, a temperature sensor, a pH sensor, a blood sugar sensor, a blood oxygen sensor, a motion sensor, a flow sensor, a velocity sensor, an acceleration sensor, a force sensor, a strain sensor, an acoustics sensor, a moisture sensor, an osmolarity sensor, a light sensor, a turbidity sensor, a radiation sensor, an electromagnetic field sensor, a chemical sensor, an ionic sensor, and an enzymatic sensor.

According to still further features in the described preferred embodiments the first acoustic activatable transducer is capable of transmitting an identification code identifying the transducer.

According to yet another aspect of the present invention there is provided a method for non-invasive monitoring of a physiological condition within a patient's body, the method comprising the steps of (a) sensing at least one parameter associated with the physiological condition via at least one sensor implanted within the patient's body to thereby obtain information pertaining to the physiological condition as an electrical output; (b) converting the electrical output into an acoustic signal via an acoustic transducer and thereby acoustically relaying the information to outside the patient's body; and (c) relaying an acoustic interrogation signal from outside the patient's body for activating the at least one sensor.

According to still another aspect of the present invention there is provided a method for non-invasive monitoring and alleviating of a physiological condition within a patient's body, the method comprising the steps of (a) sensing at least one parameter associated with the physiological condition via at least one sensor implanted within the patient's body to thereby obtain information pertaining to the physiological condition as an electrical output, (b) converting the electrical output into an acoustic signal via an acoustic transducer and thereby acoustically relaying the information to outside the patient's body; and (c) relaying an acoustic interrogation signal from outside the patient's body for activating the at least one sensor and further for activating a shunt for alleviating the physiological condition.

The present invention successfully addresses the shortcomings of the presently known configurations by providing a biosensor which can be used for non-invasive monitoring of body parameters, which does not require wiring, which does not require an integral power source, which can be effectively positioned at any location and depth within the body and which is much less subject to interrogation positional effect as compared with prior art devices.

BRIEF DESCRIPTION OF THE DRAWINGS

10 The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. 1a is a longitudinal cross section of a transducer element according to the present invention taken along lines A—A in FIGS. 2a-2e;

FIG. 1b is a longitudinal cross section of a transducer element according to the present invention taken along lines B—B in FIGS. 2a-2e;

FIG. 2a is a cross section of a transducer element according to the present invention taken along line C—C in FIG. 1a;

FIG. 2b is a cross section of a transducer element according to the present invention taken along line D—D in FIG. 1a;

FIG. 2c is a cross section of a transducer element according to the present invention taken along line E—E in FIG. 1a;

FIG. 2d is a cross section of a transducer element according to the present invention taken along line F—F in FIG. 1a;

FIG. 2e is a cross section of a transducer element according to the present invention taken along line G—G in FIG. 1a;

FIG. 3 shows the distribution of charge density across a piezoelectric layer of a transducer element resulting from the application of a constant pressure over the entire surface of the layer;

FIG. 4 shows the results of optimization performed for the power response of a transducer according to the present invention;

FIG. 5 shows a preferred electrode shape for maximizing the power response of a transducer according to the present invention;

FIG. 6 is a longitudinal section of another embodiment of a transducer element according to the present invention capable of functioning as a transmitter;

FIGS. 7a-7f are schematic views of possible configurations of transmitters according to the present invention including parallel and anti-parallel electrical connections for controllably changing the mechanical impedance of the piezoelectric layer;

FIG. 8 is a longitudinal section of a transmitter element according to the present invention including an anti-parallel electrical connection;

FIG. 9 is a longitudinal section of another embodiment of a transmitter element according to the present invention;

FIG. 10 is a block diagram depicting the intrabody and extracorporeal components of the biosensor system according to the present invention;

FIG. 11 is a schematic depiction of components of the biosensor system according to one embodiment of the present invention;

FIG. 12 is a longitudinal section of a shunt system including an acoustic transducer and pressure sensors according to another embodiment of the present invention;

FIG. 13 is a schematic depiction of the transducer and pressure sensors of FIG. 12 isolated from the shunt; and

FIG. 14 is a block diagram of the extracorporeal station components according to the present invention implemented within a helmet.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of an intrabody bio-sensing system and method which can be used for both monitoring and alleviating physiological conditions within a patient's body. Specifically, the biosensor system and method of the present invention incorporates an active acoustic transducer communicating with sensors and optionally with a shunt implanted within the patient's body for monitoring and alleviating, for example, intra-cranial pressure of a patient suffering from hydrocephalus.

The principles and operation of an implantable biosensor system according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting. For purposes of better understanding the system and method according to the present invention, as illustrated in FIGS. 10-14 of the drawings, reference is first made to the construction and operation of a transducer as described in U.S. patent application Ser. No. 09/300,553.

Referring now to the drawings, FIGS. 1a, 1b and 2a-2e illustrate a preferred embodiment of a transducer element according to the present invention which is referred to herein as transducer element 1. Transducer element 1 serves for converting received acoustic signals into electrical power and for converting electrical power to transmitted acoustic signals. As shown in the figures, the transducer element 1 includes at least one cell member 3 including a cavity 4 etched into a substrate and covered by a substantially flexible piezoelectric layer 2. Attached to piezoelectric layer 2 are an upper electrode 8 and a lower electrode 6, the electrodes for connection to an electronic circuit.

The substrate is preferably made of an electrical conducting layer 11 disposed on an electrically insulating layer 12, such that cavity 4 is etched substantially through the thickness of electrically conducting layer 11.

Electrically conducting layer 11 is preferably made of copper and insulating layer 12 is preferably made of a polymer such as polyimide. Conventional copper-plated polymer laminate such as KAPTON™ sheets may be used for the production of transducer element 1. Commercially available laminates such as NOVACLAD™ may be used. Alternatively, the substrate may include a silicon layer, or any other suitable material. Alternatively, layer 11 is made of a non-conductive material such as PYRALIN™.

Preferably, cavity 4 is etched into the substrate by using conventional printed-circuit photolithography methods. Alternatively, cavity 4 may be etched into the substrate by using VLSI/micro-machining technology or any other suitable technology.

Piezoelectric layer 2 may be made of PVDF or a copolymer thereof. Alternatively, piezoelectric layer 2 is made of a

substantially flexible piezoelectric. Preferably, piezoelectric layer 2 is a poled PVDF sheet having a thickness of about 9-28 μ m. Preferably, the thickness and radius of flexible layer 2, as well as the pressure within cavity 4, are specifically selected so as to provide a predetermined resonant frequency. When using the embodiment of FIGS. 1a and 1b, the radius of layer 2 is defined by the radius of cavity 4.

By using a substantially flexible piezoelectric layer 2, the invention described in U.S. patent application Ser. No. 09/300,553 allows to provide a miniature transducer element whose resonant frequency is such that the acoustic wavelength is much larger than the extent of the transducer. This enables the transducer to be omnidirectional even at resonance, and further allows the use of relatively low frequency acoustic signals which do not suffer from significant attenuation in the surrounding medium.

Prior art designs of miniature transducers, however, rely on rigid piezoelectric usually operating in thickness mode. In such cases the resonant frequency relates to the size of the element and speed of sound in the piezoelectric, and is higher by several orders of magnitude.

The invention described in U.S. patent application Ser. No. 09/300,553 provides a transducer which is omnidirectional, i.e., insensitive to the direction of the impinging acoustic rays, thereby substantially simplifying the transducer's operation relative to other resonant devices. Such a transducer element is thus suitable for application in confined or hidden locations, where the orientation of the transducer element cannot be ascertained in advance.

According to a specific embodiment, cavity 4 features a circular or hexagonal shape with radius of about 200 μ m. Electrically conducting layer 11 preferably has a thickness of about 15 μ m. Cell member 3 is preferably etched completely through the thickness of electrically conducting layer 11. Electrically insulating layer 12 preferably features a thickness of about 50 μ m. The precise dimensions of the various elements of a transducer element according to the invention described in U.S. patent application Ser. No. 09/300,553 may be specifically tailored according to the requirements of the specific application.

Cavity 4 preferably includes a gas such as air. The pressure of gas within cavity 4 may be specifically selected so as to predetermine the sensitivity and ruggedness of the transducer as well as the resonant frequency of layer 2.

As shown in FIG. 2b, an insulating chamber 18 is etched into the substrate, preferably through the thickness of conducting layer 11, so as to insulate the transducer element from other portions of the substrate which may include other electrical components such as other transducer elements etched into the substrate. According to a specific embodiment, the width of insulating chamber 18 is about 100 μ m. As shown, insulating chamber 18 is etched into the substrate so as to form a wall 10 of a predetermined thickness enclosing cavity 4, and a conducting line 17 integrally made with wall 10 for connecting the transducer element to another electronic component preferably etched into the same substrate, or to an external electronic circuit.

As shown in FIGS. 1a and 1b, attached to piezoelectric layer 2 are upper electrode 8 and lower electrode 6. As shown in FIGS. 2c and 2e, upper electrode 8 and lower electrode 6 are preferably precisely shaped, so as to cover a predetermined area of piezoelectric layer 2. Electrodes 6 and 8 may be deposited on the upper and lower surfaces of piezoelectric membrane 2, respectively, by using various methods such as vacuum deposition, mask etching, printing, and the like.

As shown in FIG. 1a, lower electrode 6 is preferably made as an integral part of a substantially thin electrically conducting layer 14 disposed on electrically conducting layer 11. Preferably, electrically conducting layer 14 is made of a Nickel-Copper alloy and is attached to electrically conducting layer 11 by mechanism of a sealing connection 16. Sealing connection 16 may be made of indium. According to a preferred configuration, sealing connection 16 may feature a thickness of about 10 μm , such that the overall height of wall 10 of cavity 4 is about 20-25 μm .

As shown in FIG. 2c, electrically conducting layer 14 covers the various portions of conducting layer 11, including wall 10 and conducting line 17. The portion of conducting layer 14 covering conducting line 17 is for connection to an electronic component, as further detailed hereinafter.

According to a preferred embodiment, electrodes 6 and 8 are specifically shaped to include the most energy-productive region of piezoelectric layer 2, so as to provide maximal response of the transducer while optimizing the electrode area, and therefore the cell capacitance, thereby maximizing a selected parameter such as voltage sensitivity, current sensitivity, or power sensitivity of the transducer element.

The vertical displacement of piezoelectric layer 2, Ψ , resulting from a monochromatic excitation at angular frequency ω is modeled using the standard equation for thin plates:

$$(v^2 - \gamma^2) \kappa \nabla^2 \Psi + \gamma^2 \Psi = -\frac{3(1 - \nu^2)}{2Q\delta} P + \frac{3Z\omega(1 - \nu^2)}{2Q\delta} \Psi_{\text{ext}}$$

wherein Q is the Young's modulus representing the elasticity of layer 2; b is the half-thickness of layer 2; ν is the Poisson ratio for layer 2; γ is the effective wavenumber in the layer given by: $\gamma^2 = \omega^2(1 - \nu^2)/\rho Q b^2$, wherein ρ is the density of layer 2 and ω is the angular frequency of the applied pressure (wherein the applied pressure may include the acoustic pressure, the static pressure differential across layer 2 and any other pressure the transducer comes across); Z is the mechanical impedance resulting from the coupling of layer 2 to both external and internal media of cavity 4, wherein the internal medium is preferably air and the external medium is preferably fluid; P is the acoustic pressure applied to layer 2, and Ψ represents the average vertical displacement of layer 2.

When chamber 4 is circular, the solution (given for a single frequency component ω) representing the dynamic displacement of a circular layer 2 having a predetermined radius a , expressed in polar coordinates, is:

$$\Psi(r, \phi) = \frac{J_0(\omega r) J_1(\omega a) - J_1(\omega r) J_0(\omega a)}{2i\omega(J_0^2(\omega a) + J_1^2(\omega a))} P$$

$$I_{0,0} = J_0(\omega r) J_1(\omega a), I_{1,0} = J_1(\omega r) J_0(\omega a)$$

$$Z = \frac{P_A}{b \mu_{\text{ext}}} = \left\{ \frac{4}{3} + \frac{1}{3} \right\} \rho_{\text{ext}} b$$

wherein $\Psi(r, \phi)$ is time-dependent and represents the displacement of a selected point located on circular layer 2, the specific location of which is given by radius r and angle ϕ ; J and I are the normal and modified Bessel functions of the first kind, respectively; P_A , μ_{ext} are the air pressure within cavity 4 and the height of chamber 4, respectively; and ρ_{ext} is the density of the fluid external to cavity 4.

The first term of the impedance Z relates to the stiffness resulting from compression of air within cavity 4, and the

second term of Z relates to the mass added by the fluid boundary layer. An additional term of the impedance Z , relating to the radiated acoustic energy is substantially negligible in this example.

The charge collected between electrodes 6 and 8 per unit area is obtained by evaluating the strains in layer 2 resulting from the displacements, and multiplying by the pertinent off-diagonal elements of the piezoelectric strain coefficient tensor, e_{31} , e_{32} , as follows:

$$Q(r, \phi, \eta) = \left(e_{31} \left(\frac{\partial \Psi}{\partial x} \right) \right)^2 + \left(e_{32} \left(\frac{\partial \Psi}{\partial y} \right) \right)^2$$

wherein $Q(r, \phi, \eta)$ represents the charge density at a selected point located on circular layer 2, the specific location of which is given by radius r and angle ϕ ; x is the stretch direction of piezoelectric layer 2; y is the transverse direction (the direction perpendicular to the stretch direction) of layer 2; e_{31} , e_{32} are off-diagonal elements of the piezoelectric strain coefficient tensor representing the charge accumulated at a selected point on layer 2 due to a given strain along the x and y directions, respectively, which coefficients being substantially dissimilar when using a PVDF layer. Ψ is the displacement of layer 2, taken as the sum of the displacement for a given acoustic pressure P at frequency f , and the static displacement resulting from the pressure differential between the interior and exterior of cavity 4, which displacements being extractable from the equations given above.

The total charge accumulated between electrodes 6 and 8 is obtained by integrating $Q(r, \phi, \eta)$ over the entire area S of the electrode:

$$Q = \int_S Q(r, \phi, \eta) d\Omega$$

The capacitance C of piezoelectric layer 2 is given by:

$$C = \frac{\epsilon}{2h} \int_S d\Omega$$

wherein ϵ is the dielectric constant of piezoelectric layer 2; and h is the thickness of piezoelectric layer 2.

Accordingly, the voltage, current and power responses of piezoelectric layer 2 are evaluated as follows:

$$V = \frac{2\pi \int_S Q(r, \phi, \eta) d\Omega}{\epsilon b \int_S d\Omega}, I = 2\omega \int_S Q(r, \phi, \eta) d\Omega,$$

$$W = \frac{4\pi \left[\int_S Q(r, \phi, \eta) d\Omega \right]^2}{\epsilon b \int_S d\Omega}$$

The DC components of Q are usually removed prior to the evaluation, since the DC currents are usually filtered out. The values of Q given above represent peak values of the AC components of Q , and should be modified accordingly so as to obtain other required values such as RMS values.

According to the above, the electrical output of the transducer expressed in terms of voltage, current and power responses depend on the AC components of Q , and on the shape S of the electrodes. Further, as can be seen from the above equations, the voltage response of the transducer may be substantially maximized by minimizing the area of the

electrode. The current response, however, may be substantially maximized by maximizing the area of the electrode.

FIG. 3 shows the distribution of charge density on a circular piezoelectric layer 2 obtained as a result of pressure (acoustic and hydrostatic) applied uniformly over the entire area of layer 2, wherein specific locations on layer 2 are herein defined by using Cartesian coordinates including the stretch direction (x direction) and the transverse direction (y direction) of layer 2. It can be seen that distinct locations on layer 2 contribute differently to the charge density. The charge density vanishes at the external periphery 78 and at the center 72 of layer 2 due to minimal deformation of these portions. The charge density is maximal at two cores 74a and 74b located symmetrically on each side of center 72 due to maximal strains (in the stretch direction) of these portions.

A preferred strategy for optimizing the electrical responses of the transducer is to shape the electrode by selecting the areas contributing at least a selected threshold percentage of the maximal charge density, wherein the threshold value is the parameter to be optimized. A threshold value of 30% relates to an electrode covering the entire area of layer 2.

FIG. 4 shows the results of an optimization performed for the power response of a transducer having a layer 2 of a predetermined area. As shown in the Figure, the threshold value which provides an optimal power response is about 30% (graph b). Accordingly, an electrode which covers only the portions of layer 2 contributing at least 30% of the maximal charge density yields a maximal power response. The pertinent voltage response obtained by such an electrode is higher by a factor of 2 relative to an electrode completely covering layer 2 (graph a). The current response obtained by such electrode is slightly lower relative to an electrode completely covering layer 2 (graph c). Further as shown in the Figure, the deflection of layer 2 is maximal when applying an acoustic signal at the resonant frequency of layer 2 (graph d).

A preferred electrode shape for maximizing the power response of the transducer is shown in FIG. 5, wherein the electrode includes two electrode portions 80a and 80b substantially covering the maximal charge density portions of layer 2, the electrode portions being interconnected by mechanism of a connecting member 82 having a minimal area. Preferably, portions 80a and 80b cover the portions of layer 2 which yield at least a selected threshold (e.g. 30%) of the maximal charge density.

According to the present invention any other parameter may be optimized so as to determine the shape of electrodes 6 and 8. According to further features of the invention described in U.S. patent application Ser. No. 09/000,553, only one electrode (upper electrode 8 or lower electrode 6) may be shaped so as to provide maximal electrical response of the transducer, with the other electrode covering the entire area of layer 2. Since the charge is collected only at the portions of layer 2 received between upper electrode 8 and lower electrode 6, such configuration is operationally equivalent to a configuration including two shaped electrodes having identical shapes.

Referring now to FIG. 6, according to another embodiment chamber 4 of transducer element 1 may contain gas of substantially low pressure, thereby conferring a substantially concave shape to piezoelectric membrane 3 at equilibrium. Such configuration enables to further increase the electrical response of the transducer by increasing the total charge obtained for a given displacement of layer 2. The total displacement in such an embodiment is given by: $\Psi = P_0 \Psi_{DC} + P \Psi_{AC} \cos \omega t$, wherein P_0 is the static pressure

differential between the exterior and the interior of cavity 4; Ψ_{DC} is the displacement resulting from P_0 ; P is the amplitude of the acoustic pressure; and Ψ_{AC} is the displacement resulting from P .

Accordingly, the strain along the x direction includes three terms as follows:

$$S_{xx} = \left(\frac{\partial \Psi}{\partial x} \right) = P_0 \left(\frac{\partial \Psi_{DC}}{\partial x} \right) + P \left(\frac{\partial \Psi_{AC}}{\partial x} \right) \cos^2 \omega t + 2P_0 P \frac{\partial \Psi_{DC}}{\partial x} \frac{\partial \Psi_{AC}}{\partial x} \cos \omega t$$

wherein the DC component is usually filtered out.

Thus, by decreasing the pressure of the medium (preferably air) within cavity 4 relative to the pressure of the external medium (preferably fluid), the value of P_0 is increased, thereby increasing the value of the third term of the above equation.

Such embodiment makes it possible to increase the charge output of layer 2 for a given displacement, thereby increasing the voltage, current and power responses of the transducer without having to increase the acoustic pressure P . Furthermore, such embodiment enables to further miniaturize the transducer since the same electrical response may be obtained for smaller acoustic deflections. Such embodiment is substantially more robust mechanically and therefore more durable than the embodiment shown in FIGS. 1a and 1b. Such further miniaturization of the transducer enables to use higher resonance frequencies relative to the embodiment shown in FIGS. 1a and 1b.

Preferably, a transducer element I according to the invention described in U.S. patent application Ser. No. 09/000,553 is fabricated by using technologies which are in wide use in the microelectronics industry, so as to allow integration thereof with other conventional electronic components as further detailed hereunder. When the transducer element includes a substrate such as Copper-polymer laminate or silicon, a variety of conventional electronic components may be fabricated onto the same substrate.

According to a preferred embodiment, a plurality of cavities 4 may be etched into a single substrate 12 and covered by a single piezoelectric layer 2, so as to provide a transducer element including a matrix of transducing cell members 3, thereby providing a larger energy collecting area of predetermined dimensions, while still retaining the advantage of miniature individual transducing cell members 3. When using such configuration, the transducing cell members 3 may be electrically interconnected in parallel or serial connections, or combinations thereof, so as to tailor the voltage and current response of the transducer. Parallel connections are preferably used so as to increase the current output while serial connections are preferably used so as to increase the voltage output of the transducer.

Furthermore, piezoelectric layer 2 may be completely depolarized and then repolarized at specific regions thereof, so as to provide a predetermined polarity to each of the transducing cell members 3. Such configuration enables to reduce the complexity of interconnections between cell members 3.

A transducer element according to the invention described in U.S. patent application Ser. No. 09/000,553 may be further used as a transmitter for transmitting information to a remote receiver by modulating the reflection of an external impinging acoustic wave arrived from a remote transmitter.

Referring to FIG. 6, the transducer element shown may function as a transmitter element due to the asymmetric fluctuations of piezoelectric layer 2 with respect to positive

and negative transient acoustic pressures obtained as a result of the pressure differential between the interior and exterior of cavity 4.

A transmitter element according to the present invention preferably modulates the reflection of an external impinging acoustic wave by mechanism of a switching element connected thereto. The switching element encodes the information that is to be transmitted, such as the output of a sensor, thereby frequency modulating a reflected acoustic wave.

Such configuration requires very little expenditure of energy from the transmitting module itself, since the acoustic wave that is received is externally generated, such that the only energy required for transmission is the energy of modulation.

Specifically, the reflected acoustic signal is modulated by switching the switching element according to the frequency of a message electric signal arriving from another electronic component such as a sensor, so as to controllably change the mechanical impedance of layer 2 according to the frequency of the message signal.

Preferably, a specific array of electrodes connected to a single cell member or alternatively to a plurality of cell members are used, so as to control the mechanical impedance of layer 2.

FIGS. 7a-7g illustrate possible configurations for controllably change the impedance of layer 2 of a transmitter element. Referring to FIG. 7a, a transmitter element according to the invention described in U.S. patent application Ser. No. 09/080,553 may include a first and second pairs of electrodes, the first pair including an upper electrode 49a and a lower electrode 38a, and the second pair including an upper electrode 49b and a lower electrode 38b. Electrodes 38a, 38b, 49a and 49b are electrically connected to an electrical circuit by mechanism of conducting lines 36a, 36b, 34a and 34b, respectively, the electrical circuit including a switching element (not shown), so as to alternately change the electrical connections of conducting lines 36a, 36b, 34a and 34b.

Preferably, the switching element switches between a parallel connection and an anti-parallel connection of the electrodes. A parallel connection decreases the mechanical impedance of layer 2, whereas an anti-parallel connection increases the mechanical impedance of layer 2. An anti-parallel connection may be obtained by interconnecting line 34a to 36b and line 34b to 36a. A parallel connection may be obtained by connecting line 34a to 34b and line 36a to 36b. Preferably, the switching frequency equals the frequency of a message signal arriving from an electrical component such as a sensor as further detailed hereinafter.

According to another embodiment shown in FIG. 7b, upper electrode 49a is connected to lower electrode 38b by mechanism of a conducting line 28, and electrodes 38a and 49b are connected to an electrical circuit by mechanism of conducting lines 27 and 29, respectively, wherein the electrical circuit further includes a switching element. Such configuration provides an anti-parallel connection of the electrodes, wherein the switching element functions as an on/off switch, thereby alternately increasing the mechanical impedance of layer 2.

In order to reduce the complexity of the electrical connections, layer 2 may be depolarized and then repolarized at specific regions thereof. As shown in FIG. 7c, the polarity of the portion of layer 2 received between electrodes 49a and 38a is opposite to the polarity of the portion of layer 2 received between electrodes 49b and 38b. An anti-parallel connection is thus achieved by interconnecting electrodes 38a and 38b by mechanism of a conducting line 28, and

providing conducting lines 27 and 29 connected to electrodes 49a and 49b, respectively, the conducting lines for connection to an electrical circuit including a switching element.

According to another embodiment, the transmitting element includes a plurality of transducing cell members, such that the mechanical impedance of layer 2 controllably changed by appropriately interconnecting the cell members.

As shown in FIG. 7d, a first transducing cell member 3a including a layer 2a and a cavity 4a, and a second transducing cell member 3b including a layer 2b and a cavity 4b are preferably contained within the same substrate, and layers 2a and 2b are preferably integrally made. A first pair of electrodes including electrodes 6a and 8a is attached to layer 2, and a second pair of electrode including electrodes 6b and 8b is attached to layer 2b. Electrodes 6a, 8a, 6b and 8b are electrically connected to an electrical circuit by mechanism of conducting lines 37a, 35a, 37b and 35b, respectively, the electrical circuit including a switching element, so as to alternately switch the electrical connections of conducting lines 37a, 35a, 37b and 35b, so as to alternately provide parallel and anti-parallel connections, substantially as described for FIG. 7a, thereby alternately decreasing and increasing the mechanical impedance of layers 2a and 2b.

FIG. 7e illustrates another embodiment, wherein the first and second transducing cell members are interconnected by mechanism of an anti-parallel connection. As shown in the Figure, the polarity of layer 2a is opposite to the polarity of layer 2b, so as to reduce the complexity of the electrical connections between cell members 3a and 3b. Thus, electrode 6a is connected to electrode 6b by mechanism of a conducting line 21, and electrodes 8a and 8b are provided with conducting lines 20 and 22, respectively, for connection to an electrical circuit which includes a switching element, wherein the switching element preferably functions as an on/off switch, so as to alternately increase the mechanical impedance of layers 2a and 2b.

FIG. 7f shows another embodiment, wherein the first and second transducing cell members are interconnected by mechanism of a parallel connection. As shown, electrodes 6a and 6b are interconnected by mechanism of conducting line 24, electrodes 8a and 8b are interconnected by mechanism of conducting line 23, and electrodes 6b and 8b are provided with conducting lines 26 and 28, respectively, the conducting lines for connection to an electrical circuit including a switching element. The switching element preferably functions as an on/off switch for alternately decreasing and increasing the mechanical impedance of layers 2a and 2b.

FIG. 8 shows a possible configuration of two transducing cell members etched onto the same substrate and interconnected by mechanism of an anti-parallel connection. As shown in the Figure, the transducing cell members are covered by a common piezoelectric layer 2, wherein the polarity of the portion of layer 2 received between electrodes 6a and 8a is opposite to the polarity of the portion of layer 2 received between electrodes 6b and 8b. Electrodes 8a and 8b are bonded by mechanism of a conducting line 9, and electrodes 6a and 6b are provided with conducting lines 16 for connection to an electrical circuit.

Another embodiment of a transmitter element according to the present invention is shown in FIG. 9. The transmitter element includes a transducing cell member having a cavity 4 covered by a first and second piezoelectric layers, 50a and 50b, preferably having opposite polarities. Preferably, layers 50a and 50b are interconnected by mechanism of an insu-

lating layer 52. Attached to layer 50a are upper and lower electrodes 44a and 42a, and attached to layer 50b are upper and lower electrodes 44b and 42b. Electrodes 44a, 42a, 44b and 42b are provided with conducting lines 54, 55, 56 and 57, respectively, for connection to an electrical circuit.

It will be appreciated that the above descriptions are intended only to serve as examples, and that many other embodiments are possible within the spirit and the scope of invention described in U.S. patent application Ser. No. 09/300,533.

As is detailed hereinafter, in preferred embodiments, the present invention exploits the advantages of the acoustic transducer described hereinabove and in U.S. patent application Ser. No. 09/300,533.

Thus, according to the present invention there is provided an implantable biosensor system, which is referred to hereinafter as biosensor 100.

Biosensor 100 is implantable within a patient's body for monitoring a physiological condition therein. In the course of its operation, biosensor 100 relays, on command, information in the form of acoustic signals pertaining to a parameter or parameters associated with the physiological condition as these are sensed by an implanted sensor or sensors. Furthermore, biosensor 100 according to the present invention is designed to be energized via an external acoustic interrogation signal.

As such, biosensor 100 is wire and/or integral power source independent. In addition, since the human body is, in effect, a water body and further since acoustic radiation is readily propagatable, if so desired, within water bodies in all directions, biosensor 100 of the present invention provides advantages over the prior art in terms of effective implantable depth within the body and further in terms of interrogation signal positional effect.

As further detailed hereinafter, according to a preferred embodiment of the present invention biosensor system 100 incorporates a shunt for alleviating a monitored physiological condition.

As shown in FIG. 10, and according to one embodiment of the present invention, when implanted in a monitoring or treatment intra body site, biosensor 100 of the present invention is employed for sensing or monitoring one or more parameters of a physiological condition within the patient and for transmitting acoustic signals representative of this physiological condition or these parameters out of the patient's body.

According to this embodiment of the present invention, biosensor 100 includes one or more sensors 112 for sensing, monitoring or measuring one or more parameters of the physiological conditions of the patient.

Biosensor 100 also includes an acoustic activatable transducer 114. Transducer 114 serves for receiving electrical signals from sensors 112 and for converting such electrical signals into acoustic signals. Transducer 114 also serves for receiving externally generated acoustic interrogation signals and for converting such acoustic energy into electrical power which is used for energizing sensors 112 and for rendering biosensor 100 wire and integral power source independent.

As further shown in FIG. 10, transducer 114 includes a receiving assembly 117 and a transmitting assembly 118, preferably both are integrated into a single transceiver assembly.

According to a preferred embodiment of the present invention receiving assembly 117 and transmitting assembly 118 are assembled of transducer element 1, the construction of which is further detailed hereinabove with regards to

FIGS. 1a, 1b and 2a-2c. Alternatively, a plurality of transducer elements 1 can also be utilized in various configurations (as shown in FIGS. 7b-f, 8 and 9 hereinabove) in the receiving assembly 117 and transmitting assembly 118 of biosensor 100 of the present invention.

The components of transducer 114 can be formed from separate transducer element 1 units, although the integration of one transducer element 1 into a transceiver is preferred, due to the high degree of miniaturization required in bio-sensing devices.

According to a preferred embodiment of the present invention signals received and/or transmitted by biosensor 100 are processed by a processor 113. Electrical signals generated by sensors 112 are processed through processor 113 and are forwarded in their processed or converted form to transducer 114. In addition, acoustic signals received by transducer 114 and which are converted to electrical signals (and power) thereby, are preferably further processed by processor 113.

To this end, processor 113, preferably includes a conditioner 116 and, when necessary, a digitizer 119 for processing the electrical signals received thereby from sensors 112 and/or transducer 114.

The acoustic interrogation signal is generated by an extracorporeal station 130 which includes an interrogator 135 and which is also illustrated in FIG. 10, the operation and construction of which is described in further detail below.

Sensors 112 are operable for monitoring or detecting one or more physiological conditions within the patient's body, such as the pressure and/or the temperature of the cerebrospinal fluid in the cavities or ventricles of the patient's brain. Sensors 112 then generate sensor signals representative of these measured physiological parameters. The sensor signals are typically electrical analog signals but may also be digital, depending on the type of sensor employed. It will be appreciated that sensors having a built-in analog-to-digital converter are well known in the art.

Sensors 112 are preferably conventional in construction and may include, for example, pressure sensors, temperature sensors, pH sensors, blood sugar sensors, blood oxygen sensors, or any other type of physiological sensing, monitoring or measuring devices responsive to, for example, motion, flow, velocity, acceleration, force, strain, acoustics, moisture, osmolarity, light, turbidity, radiation, electromagnetic fields, chemicals, ionic, or enzymatic quantities or changes electrical and/or impedance.

Examples of these and other sensor devices useful in context of the present invention are described in detail in the AIP Handbook of Modern Sensors by Jacob Fraden, hereby incorporated by reference.

In a preferred embodiment, sensors 112 are pressure sensor transducers such as the PVDF sensors described in U.S. patent application Ser. No. 09/161,658, which is incorporated herein by reference, or the MPK3000 series pressure sensors distributed by Moturo.

As mentioned above according to a preferred embodiment of the present invention transducer 114 is electrically coupled to sensors 112 through processor 113. Processor 113 conditions the sensor signals via conditioner 116, converts the sensor signals to a digital form (when so required) via digitizer 119, and provides the processed or converted signal to transducer 114. Upon a command, transducer 114 converts the processed electrical signals into corresponding acoustic signals which are concomitantly transmitted out of the patient's body, when subjected to an acoustic interrogation signal from station 130.

In more detail, processor 113 is electrically connected to sensors 112 and both share a common miniature substrate such as is customary in the VLSI (Very Large Scale Integration) industry. Processor 113 directly receives sensors' 112 signals by, e.g., the shortest possible wiring.

Processor 113 serves several functions. As already mentioned, processor 113 conditions via conditioner 116 the signals received from sensors 112. Such conditioning is necessary due to the miniature size and small capacitance of sensors 112, and as such, conditioner 116 provides not only appropriate amplification and filtering, but also impedance reduction, so as to substantially reduce noise pickup and thereby improve the signal-to-noise ratio of biosensor 100.

In addition, digitizer 119 is employed in processor 113 to convert the analog signals to digital signals and format the digitized signals as a binary data stream for transmission out of the patient by transducer 114 acoustic signals, which are received and interpreted by extracorporeal station 130.

Processor 113 is also operable for coding and formatting a unique device identification number for transmission with the sensors' signals for use in identifying a specific transducer 114 and/or sensor 112.

Preferably, processor 113 can be programmed to analyze the monitored signals before transmitting the signals out of the patient's body. To this end, processor 113 can be provided with a memory device and a programmable microprocessor. Many more tasks which are applicable to biosensor system 100 of the present invention can be provided by processor 113, such as, for example, calculating a reading by correlating information derived from a plurality of sensors 112.

For example, if biosensor 100 is provided with a pressure sensor and a temperature sensor for measuring both the pressure and temperature of the cerebrospinal fluid in the patient's brain, processor 113 can then be programmed to adjust the pressure signal transmitted out of the patient's body to compensate for higher or lower temperature readings as sensed by the temperature sensor and vice versa, thereby providing more accurate readings.

It will, however, be appreciated by one ordinarily skilled in the art that sole or additional/supplementary processing can be effected by processors present in extracorporeal station 130.

Preferably, transmitting assembly 118 of transducer 114 employs modulations or other methods in modifying the transmitted acoustic signal, such modulation methods are well known in the art and are described in detail in, for example, U.S. Pat. No. 5,619,997 which is incorporated herein by reference.

Extracorporeal station 130 is located outside the patient's body and is designed for powering or energizing transducer 114 of biosensor 100 which is implanted within the patient's body, and for receiving the sensors' acoustic signals.

As illustrated in FIGS. 10-11, according to one embodiment of the present invention and as further detailed in the following sections, transducers 321 of station 130 are mounted within a helmet 310. Transducers 321 are coupled via wiring with a signal generator 126, a power amplifier 128, a modulator 132, a demodulator 133, a signal conditioner 134 and a recording and analyzing device 138.

Signal generator 126 and power amplifier 128 provide energy to extracorporeal transducer 321 for generating acoustic signals which propagate from the surface into the patient's body and energize intrabody acoustic transducer 114 when impinging thereon. Signal generator 126 and power amplifier 128 may be of any known type, including devices constructed in accordance with "Data Transmission

from an Implantable Biosensor by Load-Shift Keying Using Circuit Configuration Modulator" by Zhengman Tang, Brian Smith, John H. Schild, and P. Hunter Peckham, IEEE Transactions on Biomedical Engineering, vol. 42, No. 5, May, 1995, pp. 524-528, which is incorporated herein by reference.

As already mentioned, transducers 321 are preferably of a type functionally similar to transducer element 1, the construction of which is further described hereinabove in FIGS. 1a, 1b, 2a-2b, 7b-f, 8 and 9, each of which can serve as a transmitter, receiver or a transceiver, and are preferably constructed to comply with NCRP 113: Exposure criteria for medical diagnostic ultrasound 1992, parts I and II, provided that transducers 321 when serve as a powering transmitter is capable of transmitting sufficient energy in the form of an acoustic signal for energizing biosensor 100. Preferred transducers 321 include commercial piston type transducers.

Transducers 321 are electrically connected to power amplifier 128 and acoustically communicable with transducer 114. Transducers 321 transform and deliver the energy generated by generator 126 and power amplifier 128 to transducer 114 via the body of the patient, which serves in this respect as a water body.

Demodulator 133 is operatively coupled to transducers 321 and is provided for extracting digital data received thereby from transducer 114. An example of a demodulator 133 that can be used in interrogator 115 of extracorporeal station 130 is the MC1496 or MC1596 type demodulator distributed by Motorola.

Signal conditioner 134 is connected to demodulator 133 for converting the demodulated data to a format suitable for recording or storing in external devices. An example of a signal conditioner 134 that can be used in station 130 of the present invention is the ADM202 type conditioner distributed by Analog Devices. Signal conditioner 134 may be connected with conventional recording and/or analyzing devices such as computers, printers, and displays for recording, presenting and/or further analyzing the signals transmitted by biosensor 100.

Thus, and according to this embodiment of the present invention, biosensor 100 described hereinabove is implanted in a patient for sensing, monitoring or detecting one or more parameters associated with a physiological condition of the patient. When it is desired to collect information from the body of the patient, a control console 124 commands interrogator 115 to trigger an energizing signal output from signal generator 126. The energizing signal is then modulated with other commands originating from control console 124 that governs processor 113 of biosensor 100 and multiplexer-demultiplexer 381. The modulated signal is amplified by power amplifier 128 and sent to transducer 321 to energize and render biosensor 100 operative via transducer 114 thereof. The energy thus provided through the body of the patient is also used to provide transducer 114 with energy to produce an acoustic signal related to the information thus collected by sensors 112. To this end, transducers 321 of station 130 are placed in intimate physical contact with a portion of the patient's body preferably in which biosensor 100 is implanted. Station 130 generates an acoustic interrogation signal via transducers 321 for powering biosensor 100 and for retrieving via transducers 114 sensors' 112 signals as an acoustic signal generated by transducer 114. Interrogator 115 then demodulates sensors' 112 signals and delivers the signals to recording and analyzing device 138.

It will be appreciated that in cases where each of sensors 112 provides information pertaining to a specific parameter, specific information from each of sensors 112 can be

accessed by station 130 by providing a unique identifying code for each sensor with the acoustic interrogation signal. Such a code would be interpreted by processor 113 to command the retrieval of information from any specific sensor of sensors 212.

Referring now to FIGS. 11-13. According to another preferred embodiment of the present invention and as best illustrated in FIG. 12, biosensor 100 further includes a shunt 202 for draining fluid from a portion of a patient's body, and a monitoring device 204 which is further detailed hereinbelow with respect to FIG. 13. According to a preferred embodiment, monitoring device 204 is embedded within the walls of shunt 202 for non-invasively monitoring the operation of shunt 202.

In more detail, shunt 202 according to this embodiment of the present invention is a cerebrospinal fluid shunt and is used for draining cerebrospinal fluid from a patient's brain, when so required. Cerebrospinal fluid shunt 202 is preferably formed of medical grade synthetic resin material and presents opposed ventricular 206 and distal 208 ends connected by a fluid passageway 205 which includes a valve 105. When shunt 202 is implanted in a patient, ventricular end 206 is positioned in a ventricular cavity of the patient's brain and distal end 208 is positioned in an organ or body cavity remote from the ventricular cavity so as to drain fluids from the patient's brain thereto.

As shown in FIG. 11, an appropriate site to drain the cerebrospinal fluid out of the brain may be the abdomen cavity. A further appropriate site for drainage is immediately after valve 105, in order to make the shunt tubing as short as possible and largely simplify the implantation thereof in surgery. Such drainage is effected via a tube 214 leading from shunt 202 to the patient's abdominal cavity. Another appropriate site for draining cerebrospinal fluid out of the patient's brain may be the patient's skull, close to the spine. In this case the drainage tube is much shorter, simplifying the implantation surgery and reducing the risk to the patient. In both case, valve 105 which forms a part of, and is operable by, biosensor 100 is preferably used for alleviating intracranial pressure via shunt 202.

As best illustrated in FIG. 12, monitoring device 204 is preferably formed or embedded within the sidewall of shunt 202.

Referring to FIG. 13, monitoring device 204 preferably includes one or more pressure sensors 212 and a transducer 214 which is electrically coupled with sensors 212. Like sensors 112, sensors 212 can include, for example, temperature sensors, pH sensors, blood sugar sensors, blood oxygen sensors, or any other type of physiological sensing, monitoring or measuring device responsive to, for example, motion, flow, velocity, acceleration, force, strain, acoustics, moisture, osmolarity, light, turbidity, radiation, electricity, electromagnetic fields, chemicals, ionic, or enzymatic quantities or changes.

According to a preferred embodiment of the present invention, sensors 212 are provided for sensing the pressure of the cerebrospinal fluid in shunt passageway 205 and are preferably spaced a distance apart from one another for sensing pressure at different points within passageway 205. Sensors 212 may be placed anywhere within shunt 202 and may include piezoelectric or piezo-resistive transducers, silicon capacitive pressure transducers, variable-resistance laminates of conductive ink, variable conductance elastomeric devices, strain gauges or similar types of pressure sensitive devices.

Transducer 214 is also preferably formed or embedded within the sidewall of the shunt 202 and is coupled with

sensors 212 for directly or indirectly (via a processor) receiving electrical pressure signals therefrom.

According to this embodiment of the present invention biosensor 100 which includes monitoring device 204 is implanted in a patient as illustrated generally in FIG. 11 for draining or removing cerebrospinal fluid from the patient's brain for treating hydrocephalus. Monitoring device 204 which is preferably formed within the sidewalls of shunt 202 senses or detects the pressure of the cerebrospinal fluid within shunt 202 and delivers pressure signals to transducer 214. Preferably such monitoring is performed by sensors 212 periodically. Such periodic readings can be stored and processed within a processor for later access.

When it is desired to collect information from sensors 212, station 130 (or at least transducers 321 thereof) is placed adjacent a portion of the patient's body in which biosensor 100 is implanted. As described before, station 130 generates an interrogation signal delivered through transducers 321 for concomitantly powering biosensor 100 and retrieving data therefrom via transducer 214 in a fashion similar to as described above with respect to transducer 114. Should the data collected indicate an abnormal intracranial pressure, valve 105 of shunt 202 is opened to drain cerebrospinal fluid therethrough. To this end station 130 can be commanded to provide power for the opening of valve 105. This operation can be controlled either manually or by a preprogrammed processor.

According to another preferred embodiment of the present invention and as shown in FIGS. 11 and 14 there is provided a transducing assembly 351 which forms a part of station 130. In one configuration, as best seen in FIG. 11, assembly 351 is incorporated into a helmet 310. Helmet 310 includes a plurality of transducers 321, each may serve as a transmitter, receiver or transceiver, positioned at various locations so as to provide full transmittance/reception spatial coverage of the brain volume.

As shown in FIG. 11, a cable bundle 350 physically connects assembly 351 to multiplexer/demultiplexer 381, which is computer controlled. Multiplexer/demultiplexer 381 serves several functions, including (i) providing a transmittance signal to transducers 321 from power amplifier 128, (ii) conveying sensors' 112 or 212 signals from the body to signal conditioner 134, (iii) providing a computer-controlled multiplexing for transducers 321 when used as transmitters; (iv) providing multiplexing for transducers 321 when used as receivers; and/or (v) providing decoupling between the high power transmission signals from amplifier 128 and the low amplitude signals received from transmitting assembly 118 which is located within the body, into signal conditioner 134. It will be appreciated that multiplexer/demultiplexer 381 both isolates and routes the transmitted and received signals.

According to a preferred embodiment of the present invention the operation of assembly 351 included within helmet 310 is effected following pre calibration of the required location of the transducers over the helmet by, preferably, applying a method which is based on a positioning model.

Such a positioning model allows for an accurate placement of the extracorporeal transducers such that accurate insouflying of the brain volume is provided at an approximately uniform level throughout.

In addition, to achieve such uniformity a three dimensional acoustic propagation model of the skull and brain can also be applied.

Employment of wide beam low frequency ultrasonic transducers may be advantageous in providing an economical coverage.

In addition, focusing the acoustic beams of the extracorporeal transducers on the intrabody transducer is also advantageous because in such cases narrow beam transducers of low frequency ultrasound can be efficiently utilized.

Thus, for appropriately positioning such extracorporeal transducers, either a positioning model or a converging (in-line) spheroidal acoustic array model with scattering can be used to provide the positional information required. With each of the transducers configuration envisaged above, a first run calibration session is employed in which communication between the helmet (extracorporeal) transducers and the intrabody transducer is tested for maximal accuracy.

The present invention is advantageous over the existing art because it employs acoustic signals which are more readily propagable in water bodies, such as the human body, as compared to radio frequency signals.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

What is claimed is:

1. An implantable biosensor system for monitoring a physiological condition in a patient, the biosensor system comprising:

(a) at least one sensor for sensing at least one parameter of a physiological condition and for generating an electrical sensor signal representative of the physiological condition; and

(b) at least one first acoustic activatable transducer being directly or indirectly coupled with said at least one sensor, said at least one first acoustic activatable transducer being for converting a received acoustic interrogation signal from outside the patient's body into an electrical power for energizing said at least one sensor, said at least one first acoustic activatable transducer further being for converting said electrical sensor signal of said at least one sensor into an acoustic signal receivable out of the patient's body, such that information pertaining to said at least one parameter of the physiological condition can be relayed outside the patient's body upon generation of an acoustic interrogation signal.

2. The biosensor system of claim 1, wherein said at least one first acoustic activatable transducer includes:

(i) a cell member having a cavity;

(ii) a substantially flexible piezoelectric layer attached to said cell member, said piezoelectric layer having an external surface and an internal surface, said piezoelectric layer featuring such dimensions as to enable fluctuations thereof at its resonance frequency upon impinging of said acoustic interrogation signal; and

(iii) a first electrode attached to said external surface and a second electrode attached to said internal surface.

3. The biosensor system of claim 2, wherein said piezoelectric layer is of a material selected from the group consisting of PVDF and piezoceramic.

* * * * *



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Giuffre

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[45] Date of Patent: Mar. 28, 2000

[54] VIRTUAL NEUROLOGICAL MONITOR AND METHOD

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[75] Inventor: Kenneth A. Giuffre, Wyckoff, N.J.

Primary Examiner—William E. Kamm
Attorney, Agent, or Firm—McGlow and Tuttle, P.C.

[73] Assignee: Hypervigilant Technologies, Holokus, N.J.

[21] Appl. No.: 08/970,738

[37] ABSTRACT

[22] Filed: Nov. 14, 1997

The monitor provides a means of registering and/or predicting changes in brain and central nervous system activity by processing cardiovascular monitoring data and using pattern recognition by trained computing means to predict changes in the state of the central nervous system. Hence, a "virtual" neurological monitor is created where cardiovascular data are processed through the described means and a set of real-time neurological state predictions are made.

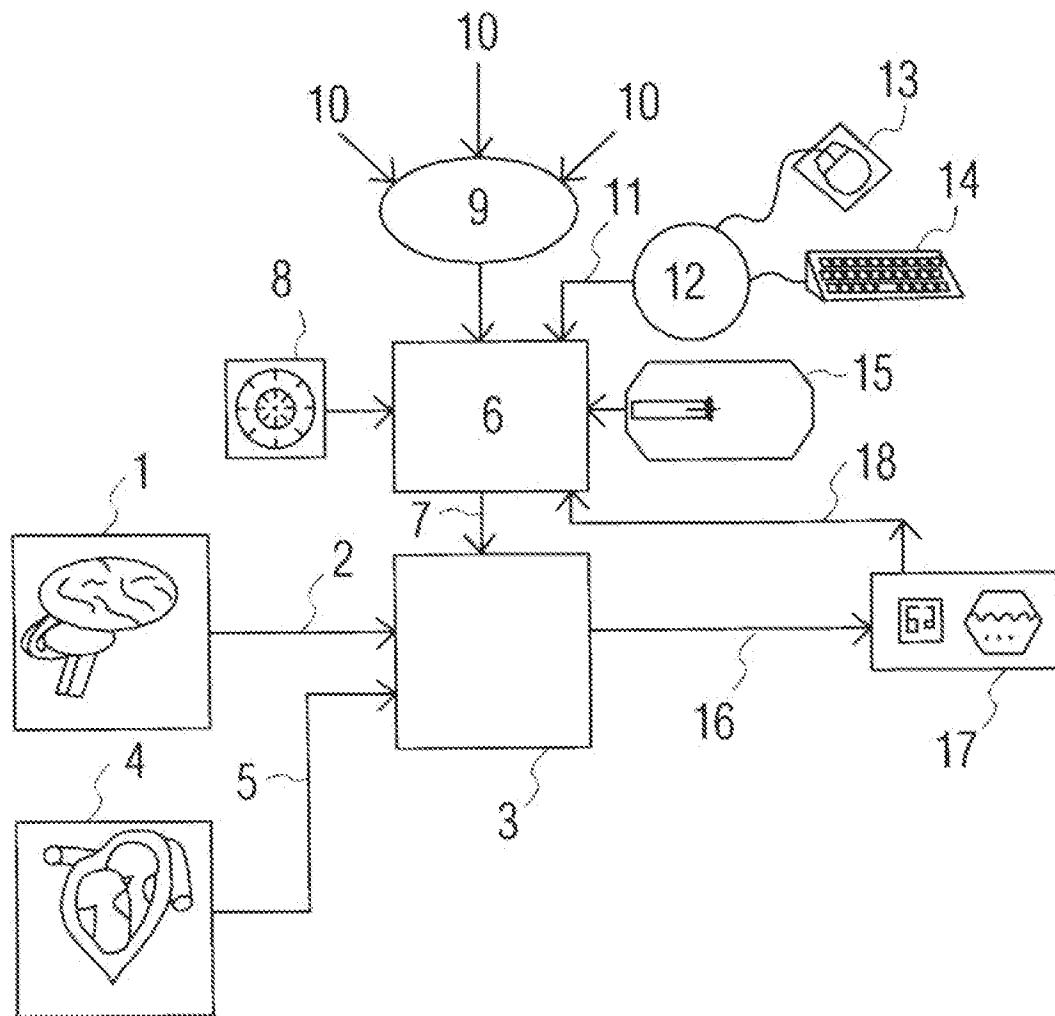
[51] Int. Cl. 7 A61B 5/0432
[52] U.S. Cl. 600/483
[58] Field of Search 600/483

[56] References Cited

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20 Claims, 6 Drawing Sheets



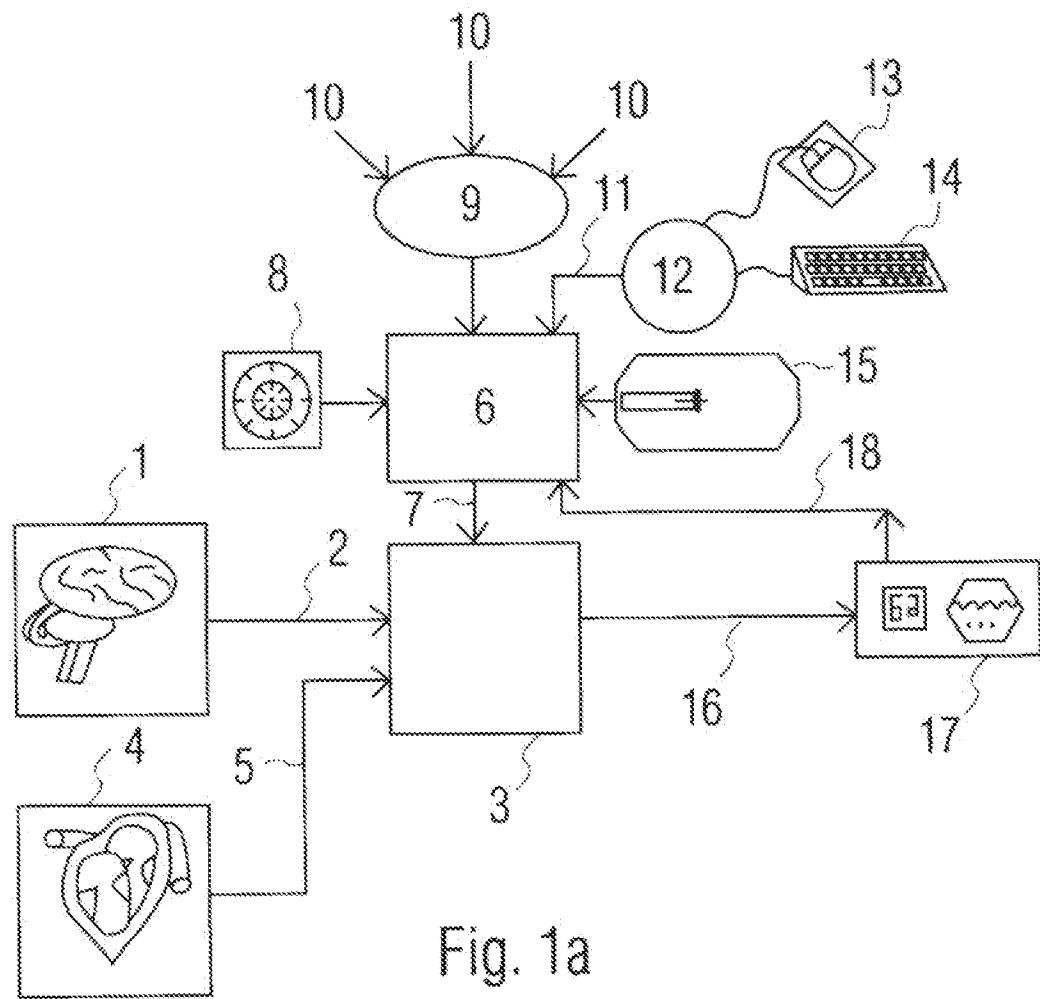
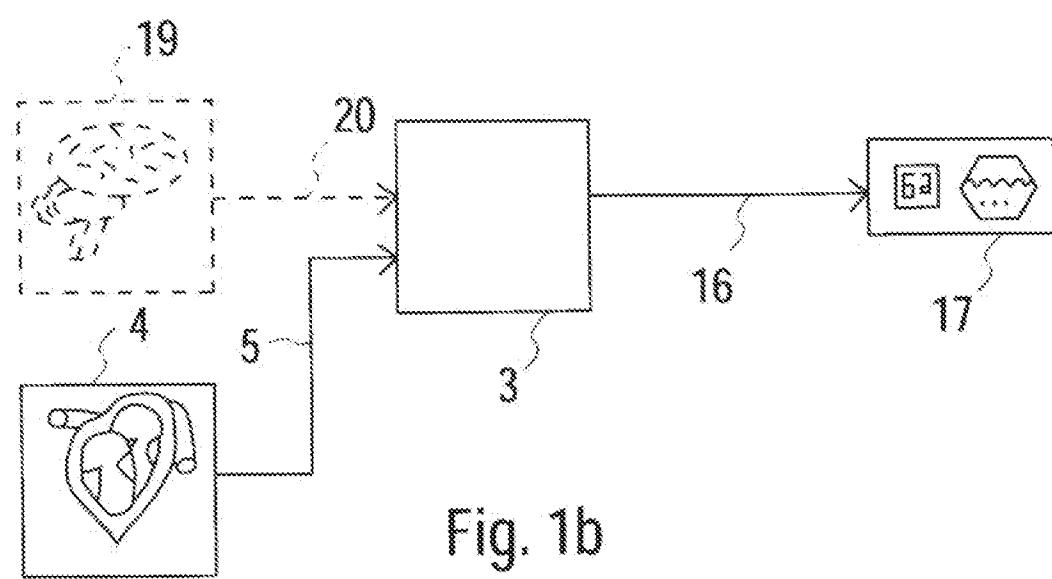


Fig. 1a



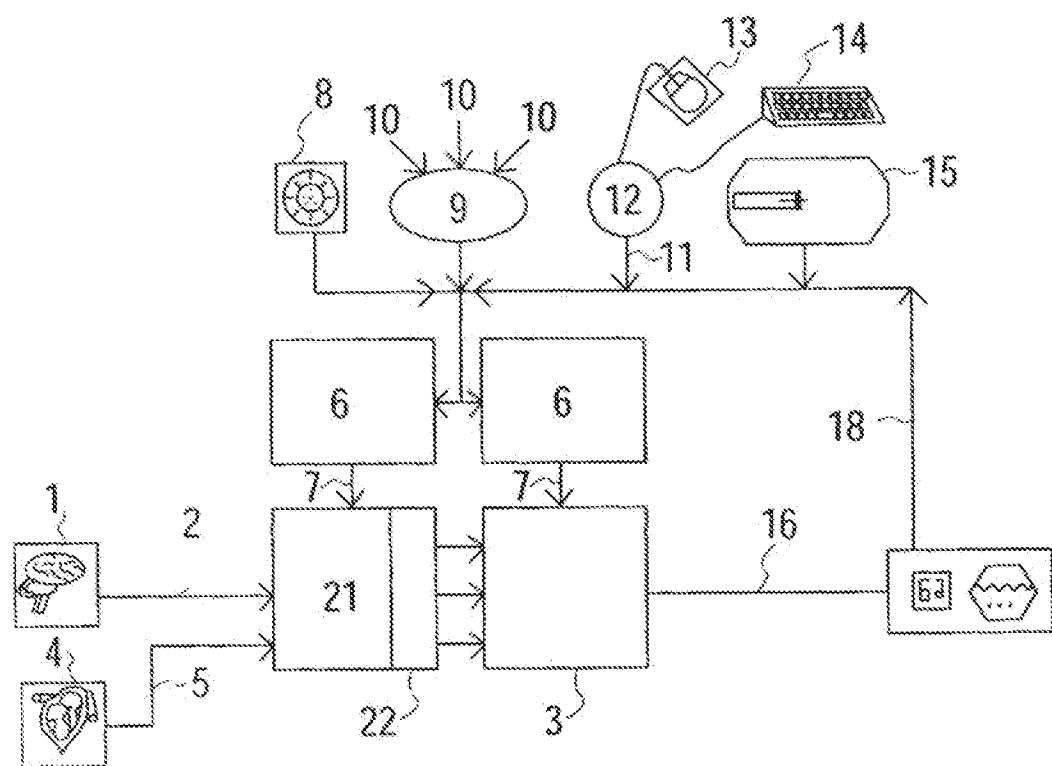
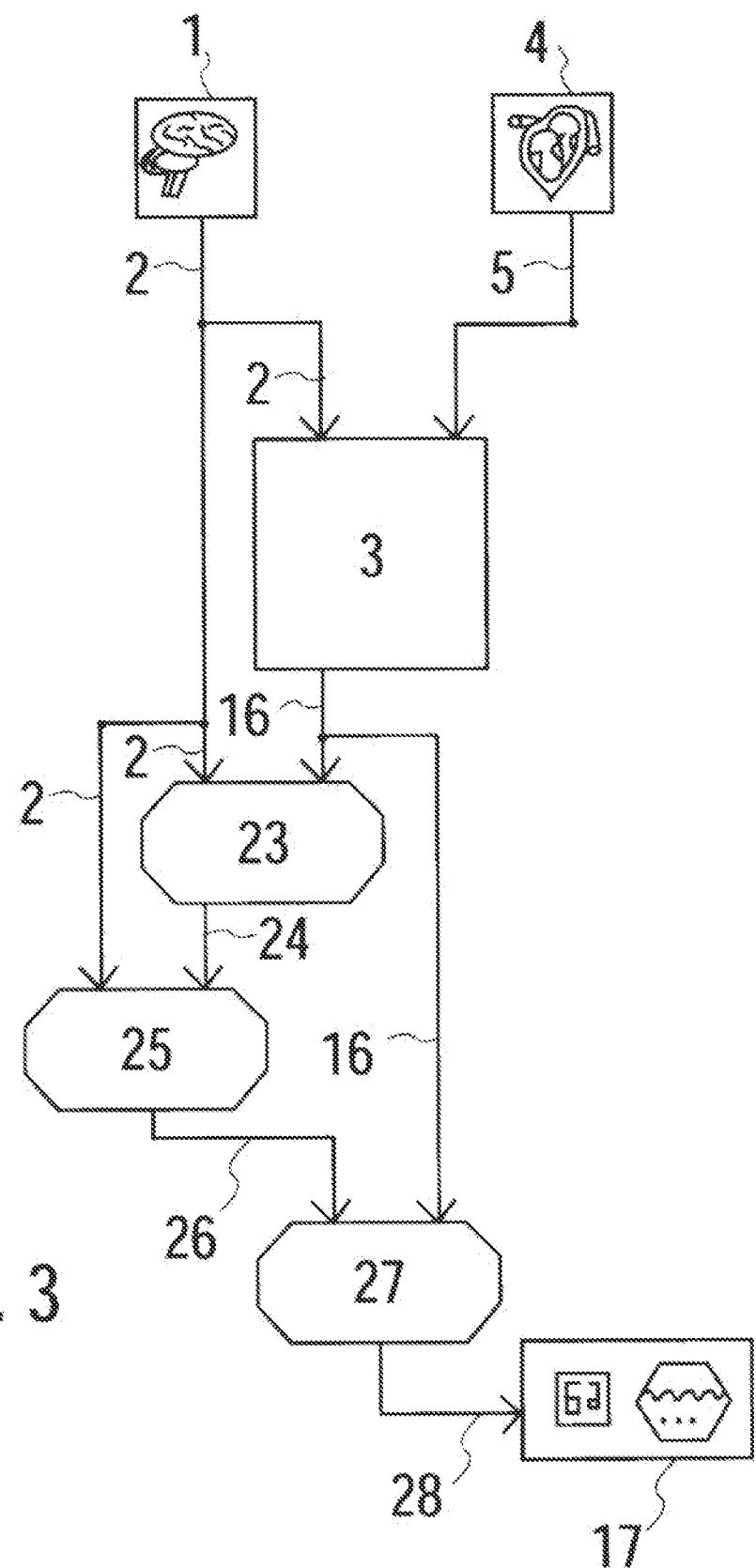
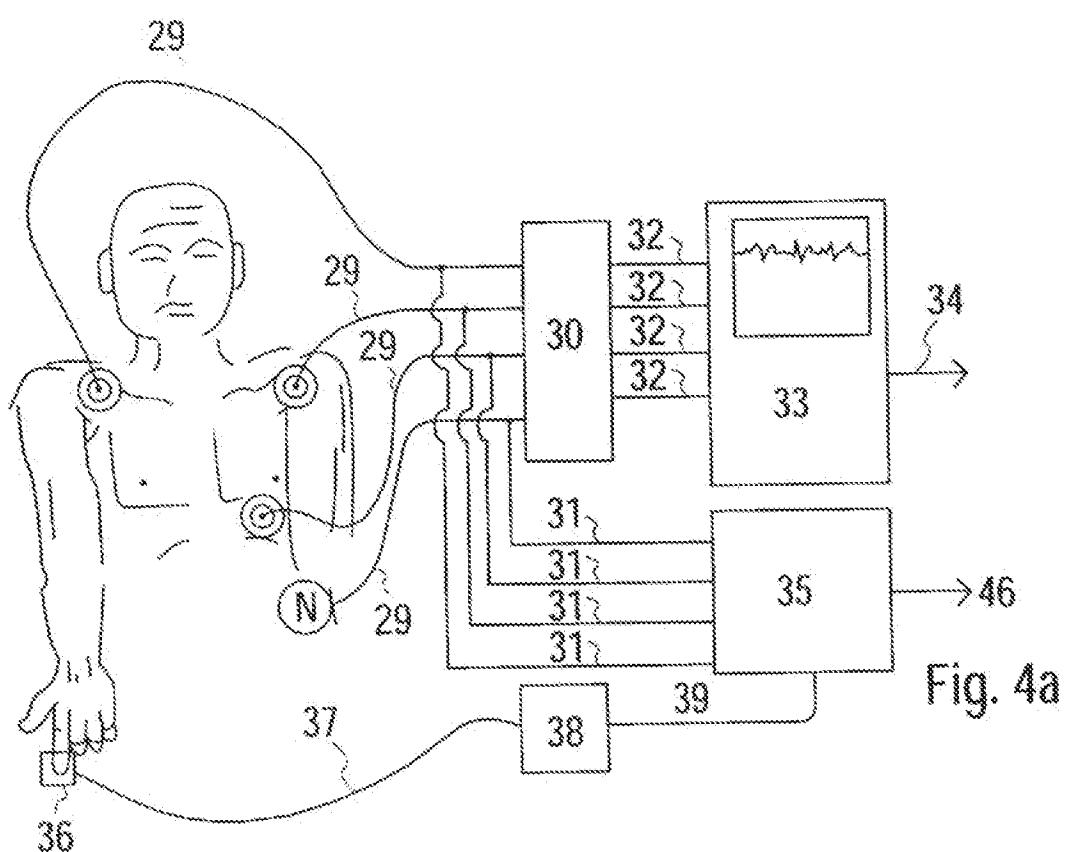
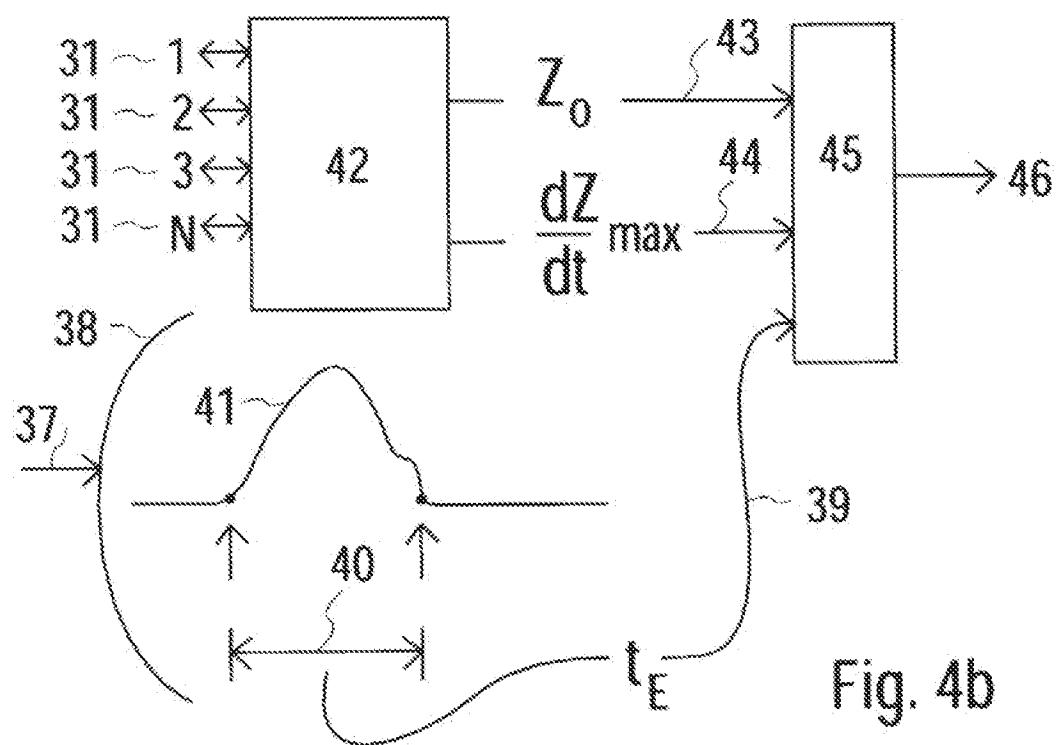


Fig. 2







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VIRTUAL NEUROLOGICAL MONITOR AND
METHOD

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to neurophysiological monitoring systems that provide clinical and/or research data on the relationship(s) between changes taking place in the central nervous system during stress, surgery, anesthesia, and other conditions resulting in dynamic brain state changes; and resulting changes seen in the autonomic nervous system and the cardiovascular system. Such systems, when noninvasive, provide less risk to subjects while guiding management, diagnosis, and treatment and/or alerting observers to changes like awareness under anesthesia, brain ischemia, pain, and severe stress.

Since neurophysiological monitoring equipment per se adds to the complexity of setup, increases the demands on the operator, raises the cost of care, and if used improperly, adds to the risk of mistaken interpretation, a system that can predict brain states using already implemented cardiovascular monitoring modalities will allow for such predictive capabilities while minimizing risk, cost, and added complexity of such a setup.

2. Description of the Prior Art

Neurological and cardiovascular physiological monitoring systems currently in use utilize a number of methods to provide observers with information on the functional states of the central nervous system, autonomic nervous system, and the cardiovascular system.

Invasive cardiovascular dynamic measurements in use include analysis of peripheral arterial pulses, pressures taken from catheters placed in the great veins, the heart chambers, the pulmonary arterial bed, and the pulmonary venous bed; continuous and intermittent thermodilutional methods of cardiac output assessment; radiotracer scanning; and continuous fiberoptic oximetric assessment of central mixed venous hemoglobin oxygen saturation.

Noninvasive cardiovascular and neurological monitoring systems employ electrophysiological measurements from the skin based on native bioelectric impulses (as in standard electroencephalography (EEG), processed EEG, standard electrocardiography (ECG), and processed ECG) or resulting from programmed stimuli to the skin or sensory organs as in somatosensory evoked potentials (SSEP), brainstem audio evoked response (BAER), visual evoked potentials (VEP), motor evoked potentials (MEP), and facial electromyography (FACI); or from programmed current passed through the body to obtain an index of bioimpedance as a means of predicting cardiovascular dynamics.

Other noninvasive systems employ plethysmographic or doppler techniques to provide a pulse waveform for analysis or utilize ultrasound in the form of echocardiography imaging, simple surface or esophageal Doppler analysis, or detect sound as in phonocardiography.

Recently, a great deal of research has been conducted into mathematical models to predict the cardiac output, left ventricle filling and ejection volumes of the heart, and stroke work of the heart, based on changes in the bioelectric impedance of the thorax to varying weak alternating electrical currents. This technique, known as thoracic bioimpedance, has been shown to accurately and noninvasively predict changes in cardiovascular dynamics in response to various stimuli including hemorrhage, shock, stress, and anesthesia (U.S. Pat. No. 5,309,917). Biboulet et.

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3. (8U.J.Anesth. 1996;76(1):81-84) for example, using the technique of thoracic bioimpedance, has shown that patients exposed to blood dilution have a much different cardiovascular response when anesthetized during said dilution. This supports the concept that bioimpedance-based cardiovascular analysis is sensitive to cardiovascular changes that occur during the transition from the awake to the anesthetized state. Thus far, bioimpedance per se has not been used as a gauge to measure level of consciousness by itself nor in combination with direct cerebral monitoring as with evoked potentials or EEG. Since subtle patterns of change in cardiovascular patterns are more telling with respect to changes in brain states, and absolute numbers less important, Giuffre and Anzano have recently proposed an improvement in existing bioimpedance modeling involving the use of a pulse or doppler plethysmographic signal from the heart itself or a peripheral artery to more accurately estimate systolic ejection time in combination with traditional bioimpedance cardiovascular modeling algorithms (Giuffre, Anzano, U.S. Patent filing November, 1977).

Several authors have also recently shown that differing levels of consciousness, stress, and anesthesia result in changes in the normal heart rate variability that occurs in response to breathing and which has been termed cardiac vagal tone (Billman et. al., *Heart Circ Physiol*, 1990; 37:H896-H902) because the vagus nerve of the parasympathetic autonomic nervous system is thought to mediate these heart rate changes, also described by Porges (*Pediatrics* 1992; 90:498-504) and which forms the basis for biophysical analysis in U.S. Pat. No. 5,510,944 and in Jaffe et. al. (*J Clin Monit* 1994; 10(1):45-48). Anesthetic depth has been shown to affect vagal tone (Ireland et. al., *Br J Anesth* 1996; 76(3):657-62), (Laison et. al., *J Clin Anesth* 1992; 4(4):265-76), (Alkire et. al., *Anesthesiology* 1997; 87(3A):A175). These methods utilize ECG or pulse measurements and thus far have not incorporated EEG-trained classification and prediction computer models into their data collection and processing.

Other methods that have attempted to reliably measure brain activity as a function of level-of-consciousness, anesthetic depth and/or state of alertness include sensors measuring microexpression changes in the face (U.S. Pat. No. 5,195,231) (Struys et. al., *Anesthesiology* 1997; 87(3A):A9), heart rate response to ocular compression (Shapiro et. al., *Psychophysiology* 1996; 33(1):54-62), contractile response of the lower esophagus (Maccioh et. al., *J Clin Monit* 1988; 5(4):247-53), and the H-reflex measuring amplitude and latency of spinal reflex arc at the tibial nerve (Magabedery et. al., *Bell Johns Hopkins Hosp* 1951;88:499) and other reflex arc responses (Chabal et. al., *Anesthesiology* 1989; 70:236-239). Electrical stimulation has been shown to even affect levels of central nervous system chemical mediators of mood and pain sensation. Low frequency peripheral electrical stimulation raises brain levels of endogenous opiates and is antagonized by opiate antagonists (Chiang, *Scientia Sinica* 1973;16:210-217, and Pomeranz, *Basis of Acupuncture*, Springer Verlag 1991:250-260). Higher frequency peripheral electrical stimulation raises brain amines like serotonin (Han, *Scientia Sinica* 1979; 22:91-104 and Lichman et. al. *Behavioral Neuroscience* 1991; 105(5):687-698). None of these brain responses to various modes of stimulation have been correlated with direct neurological monitoring data in combination with a trained computer classification and prediction model. A neural net was used in comparing hemodynamic responses to electroencephalography and facial myography (Watt et. al., *Anesthesiology* 1995;83(3A):A32, and Lang et. al., *Anesthesiology*

ogy 1994;81(3A):A177) but an attempt was made at using the hemodynamic data to back-predict the neurophysiological data.

Beyond simple, wave processed (Billard et. al., Anesthesiology 1993; 78(3A):A174), and bispectral index electroencephalography (U.S. Pat. No. 5,010,991), (Rosow et. al., Anesth Clin of NA: Annual of Anes Pharm 1998;2:89-107), (Billard et. al., Anesthesiology 1996; 85(3A):A32), other variations have been utilized to measure anesthetic depth and level of consciousness. In U.S. Pat. No. 4,869,264, the EEG response to infrared light passed through closed eyelids is utilized. Even further involvement of the body sensory means via stimulation are demonstrated in U.S. Pat. No. 4,570,640 where the body surface is stimulated, and in U.S. Pat. No. 4,201,224 where statistical Z transformations are used to process multimodal stimulation response as measured by EEG, ECG, and evoked potentials. Though this method uses a statistical predictive model, it does not attempt to create data prediction in the absence of neurological monitoring. In the method described by Muthuswamy et. al. (J Clin Monitoring 1996; 12:353-364), measurement of end tidal expired carbon dioxide is utilized in combination with processed EEG and a predictive computer algorithm. None however, have specifically combined their specific method of physiologic monitoring with neurologic monitor output to train a classification/prediction model for predicting states of central nervous system activity as a function of said physiological monitoring in the absence of neurologic monitor data.

Evoked potential monitoring has also been utilized as a measure of level of consciousness (Dai et. al., Br J Anes 1997; 78:820-84) as well as positron-emission tomography (PET) scanning (Anesthesiology 1996;85(3A):A9).

Various computer techniques have been employed in the creation of classification/prediction models for management of biophysical monitoring data. Neural network programming algorithms have been shown to be effective for recognizing patterns in biophysical monitoring modes (Baxi WG, Lancet 1995;346:1135-38). Kloppel describes the use of neural networks in EEG analysis (Neuropsychobiology 1994;29:33-38) along with Jando et. al. (Electroencephalography and Clin Neurophys 1993;86:103-109). Neural networks in EEG analysis have even been used in analyzing stages of sleep (Schudelbrand et. al., Sleep 1996; 19(1):26-35). Neural networks have also been utilized in pattern recognition of neurological evoked potential signals (Laskaris et. al., Electroencephalography and Clin Neurophys 1997;104:151-56).

Statistical methods have also been used as previously mentioned in the citation of U.S. Pat. No. 4,570,640 and in other modes of monitoring including neonatal monitoring of heart rate variability and audiology testing (both cited by Abtech Corp., Charlottesville, Va., 1995).

Newer software methods combine statistical analysis with neural net training (e.g. Model Quest software, Abtech Corp., Charlottesville, Va., 1996) or neural net training with genetic algorithms for inducing changes in neural net configuration to auto-optimize the model during training (e.g. Neuroshell Easy Predictor; Neuroshell Easy Classifier; both by Ward Systems Group, Frederick, Md., 1997).

None of the above methods combine specific methods of physiological assessment with EEG-based training of neural networks to allow prediction and assessment of either state of anesthetic depth or state of consciousness by using said EEG-trained neural net to predict the state of the central nervous system as a function of the output of said physi-

ological assessment and without the input from an EEG or other brain-based monitoring system.

SUMMARY AND OBJECTS OF THE INVENTION

According to the invention, real time neurophysiological diagnostic apparatus is provided along with a method for evaluating changes in cardiovascular dynamics and utilizing a classification and prediction computing means (e.g. statistical program, neural network, genetic algorithm, or hardwired parallel distributed processing computer) to estimate brain activity like general arousal and autonomic neural activity as a function of a training period in which data produced by one or several cardiovascular monitoring means is coupled with direct neurophysiological monitor data (EEG, evoked potentials, processed EEG et. al.; refer to claims). After such a training period, the data from the noninvasive cardiovascular monitoring means is interpreted via the trained neural net or other parallel system or self-teaching system without being coupled to the neurophysiological monitor means. Hence, the result is a noninvasive cardiovascular monitor which, using a trained pattern recognition system, estimates the neurophysiological state of the subject. Hence, the result is a cardiovascular monitoring system which, using a trained pattern recognition system, estimates the neurophysiologic state of the subject thereby acting as a "virtual" brain monitor. In the preferred embodiment, noninvasive cardiovascular monitor means capable of registering such changes in the cardiovascular system in response to central nervous system and/or autonomic system changes include thoracic bioimpedance, electrocardiography, and heart rate variability. Since a large body of biomedical research has demonstrated a direct relationship between changes in psychological states, states of arousal, states of stress, and states of consciousness with corresponding changes in the behavior of the cardiovascular system, the disclosed system can estimate brain and central nervous system activity.

The self-teaching computer can comprise a neural net software with or without genetic training algorithms (including but not limited to simple multilayer single connection nets, recurrent nets with connections to previous layers with various amounts of dampening in these back connections (Jordan-Ebner nets), multiple hidden layers with differing characteristics (Ward nets), recurrent nets where each layer is back-connected to every previous layer (Jump connection nets), unsupervised classification net models (Kohonen nets), supervised classification net models (probabilistic nets), nets involving grouped data bonding (polynomial and regression nets), and general genetic regression neural networks (GRNN)), a neural net chip with accompanying software and/or a parallel processor, alone and/or in combination with standard statistical evaluation means. Such a trained computing means acts in real-time though training can occur at slower rate to allow for specific human operator data and program manipulation, and/or incorporation of an additional genetic-type algorithm or other optimization system(s) to incrementally permit the system and/or change models until data management is optimal.

In one particular embodiment, a neural net software program is fed derived information from a thoracic bioimpedance monitoring system whose processing of systolic ejection time is improved by the use of pulse plethysmography (Giuffrè, Arizano, U.S. Patent filing November 1997); is coupled with a bispectral processed EEG system via a training protocol with data being entered through a variety

of state changes. The resultant trained neural net software model then, *setting free* of electroencephalographic input, evaluates the improved bioimpedance-measured cardiovascular state and produces an estimated neurophysiologic profile of the subject being monitored.

In the described embodiment, various neural net models can be manipulated and tested for optimal performance. Neural net architectures used can include simple nets where each input is connected to each node in a single or multiple hidden layers with various methods of connection, weighting, dampening, and learning as described herein.

The disclosed method provides a simple, noninvasive means for determining the level of arousal or depth of anesthesia of a subject, or other brain state, without requiring the use of electroencephalography or other neurophysiologic monitoring means, and hence, eliminating the need for associated electrodes attached to the head and other neural structures, the attendant cost, and the associated demands of attention and time on support staff. Instead, a real-time neural network that has been trained to associate patterns of cardiovascular biodynamics with electroencephalographic patterns, provides an estimate of the current neurophysiologic state of the patient thereby simulating the output of a neurophysiologic monitoring means, i.e., a "virtual" brain-state monitoring means.

It is an object of the invention to allow for monitoring of neurological changes without requiring the use of elaborate neural monitoring systems and their associated electrodes. Besides adding convenience and decreasing cost to the monitoring procedure, it makes the setup less complicated and hence, less error-prone. Furthermore, in situations where placement of electrodes on the head and other neural structures is impractical or difficult; states of arousal, sleep, stress, anesthesia, and brain insults like ischemia, can still be monitored.

It is a further object of the invention to make use singly or in combination, one or more cardiovascular monitoring means. Such means can include thoracic bioimpedance, electrocardiography, analysis of heart rate variability, invasive central cardiac and pulmonary arterial monitoring, pulse waveform analysis, et. al.

In embodiments where thoracic bioimpedance is utilized, standard chest bioimpedance electrodes can be used or, to simplify setup and operation while decreasing cost, a limited method of bioimpedance current and pickup can be coupled directly to standard ECG electrodes. Such data while possibly not providing optimal absolute cardiovascular baseline numbers, will register changes in bioimpedance-based cardiovascular parameters, changes being more important at providing correlation with changes in the central nervous system state than the absolute cardiovascular numbers per se. Furthermore, data provided by pulse plethysmography can refine said thoracic bioimpedance calculation models by providing for enhanced estimation of cardiac systolic ejection time (Giuffre', Anzani, U.S. patent filing November 1997).

According to one embodiment of the invention, level of consciousness of patients can be assessed by training the self-teaching computer to recognize such states through coupling said cardiovascular data to simple or processed EEG data. Other states and state changes that the self-teaching computer can be trained to include non-anesthetic sleep states for sleep monitoring; stress levels as a means of biofeedback, meditation, or lie detection; and brain ischemia or reduced cerebral bloodflow as might occur during vascular surgery, shock, or during high g-force maneuvers in aircraft or spaceflight means.

According to another embodiment of the invention, depth of anesthesia can be measured using similar methods and incorporating warning means to alert the operator of impending changes such as awakening or conversely, reaching dangerous levels of anesthetic depth. Similarly, warning means can be coupled to systems described to warn operators of impending loss of consciousness as might occur during high g-force flight maneuvers or impending brain ischemia with vascular occlusion during surgery.

It is a further object of the invention to help monitor non-anesthetic drug-induced brain changes as produced from drug overdose, drug abuse, or drug therapy as in intravenous magnesium therapy for toxic pregnant patients where magnesium acts as a brain depressant. Similarly, brain states accompanying alcohol withdrawal and drug withdrawal during rehabilitation therapy can be monitored, as well as the level of brain impairment that accompanies alcohol or drug intoxication.

It is a further object of the invention to provide easy monitoring of seizure activity as is produced during psychiatric electroconvulsive therapy.

The various features of novelty which characterize the invention are pointed out with particularity in the claims annexed to and forming a part of this disclosure. For a better understanding of the invention, its operating advantages and specific objects attained by its uses, reference is made to the accompanying drawings and descriptive matter in which preferred embodiments of the invention are illustrated.

BRIEF DESCRIPTION OF THE DRAWINGS IN THE DRAWINGS:

FIG. 1a is a block diagram depicting the relationship between cardiovascular monitor means, brain monitor means, and event data collection means, all feeding data into computer classification and prediction means which is in the training mode;

FIG. 1b is a block diagram depicting the relationship between the cardiovascular monitor means, the computer classification and prediction means, and the output means which portrays data resulting from prediction based on the trained system and resulting in an output simulating that which would be produced by brain monitor means alone, hence a "virtual" brain monitor means. Here the computer classification and prediction means has been fully or partially trained;

FIG. 2 is a block diagram depicting the computer classification and prediction means in the training mode as depicted in FIG. 1a except the data is stored and released in the computer means at a slower timed rate for less-than-real-time processing and training. Here the computer classification and prediction means is in the training mode;

FIG. 3 is a block diagram depicting a method by which the trained computer classification and prediction means can use data from cardiovascular monitor means to create a simulated brain monitor means signal which it compares with an actual brain monitor means signal, and using a series of comparator means, constructs a hybrid signal with decreased artifact;

FIG. 4a depicts a subject undergoing cardiovascular monitoring via the preferred embodiment whereby a bioimpedance cardiovascular monitoring means acts through the leads of an electrocardiography monitor whose signal is processed by a low pass filter so as not to interfere with the ECG signal. The bioimpedance monitor is specifically configured such that a pulse plethysmographic means connected to a digit on the subject aids in the processing of the

bioimpedance signal (method described by Giulire, Anzano, U.S. patent filing November 1997); and

FIG. 4b depicts the bioimpedance cardiovascular monitoring means in block format demonstrating one method by which different methods of cardiovascular monitoring can be combined, specifically in this embodiment, said Giulire Anzano method by which pulse plethysmographic signal is utilized to obtain systolic ejection time and multiplied with bioimpedance baseline impedance and impedance derivative maximum to produce accurate cardiovascular measurements.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring to the drawings in particular, FIG. 1a depicts a block diagram in which the brain of the subject is monitored via brain monitoring means 1. The brain monitoring data 2 is transferred to computer classification and prediction means 3 which is in the training mode. Simultaneously, cardiovascular data 5 is transferred from cardiovascular monitoring means 4 to computer classification and prediction means 3. To further enhance training, event parameter organizer 6 transfers event data 7 that is being recorded simultaneously, to the computer means 3 in said training mode. Event organizer 6 receives data from various sources including specific training software delivery means 15, miscellaneous event recorder 9 recording miscellaneous events 10, human interface organizer means 12 receiving data from mouse-type data input device 13, and keyboard type input device 14. Further embodiments could include voice and pen input to 12 as well, which could also convey human input data via 11 to event organizer 6 and ultimately via 7 to computer classification and prediction means 3. Events, in order to be properly organized with cardiovascular input 5 and brain input 2, are marked for time via timing means 8. Processed data from the computer means 3 is sent via 16 to virtual neuro monitor 17 which displays predicted brain monitoring data resulting from computer means 3 to the operator in a variety of forms including two dimensional moving graphic and simple numerical display(s) on virtual brain monitor 17. This output can be fed back to the event organizer means 6 for the purposes of enhancing training of the computer means 3.

The event means may be one of a variety of devices or a supply of data which is useful for the training phase or for the continued training of the monitoring system. Although a training period or phase is referred to herein, this may be ongoing or continuous. At least some initial training phase is need prior to use of the system as a monitor. Event means that are useful include one or more of: an expired gas concentration evaluation system for obtaining expired gas concentration data; a drug infusion device providing drug infusion data; input gas concentration regulation means providing input gas concentration data; means for supplying data derived from spreadsheet type data depositories; human operator input based on subjective and/or objective evaluation of the subject being monitored; expired ethanol analysis; magnesium levels monitoring means for obtaining magnesium therapy monitoring data; specific drug or alcohol blood level monitoring means to provide drug/alcohol blood level data; a standard skin galvanic resistance lie detector for providing galvanic lie detector resistance data; a meditation state source data indicating achieved states of meditation; means for monitoring specific events during surgery and providing surgery event data; g-force measurement means for providing g-force data within an aircraft, spacecraft, and/or flight simulator; and means for providing

psychometric performance data based on answers to questions and/or performance of specific tasks.

The cardiovascular monitoring means may be one or more of: a thoracic bioimpedance monitoring means; a modified thoracic bioimpedance monitoring means that concomitantly utilizes electrodes positioned on the subject and used for electrocardiographic analysis; a heart rate variability real-time time series analysis device; a electrocardiographic morphology analysis device operating in real time and producing interpretive data; a electrocardiographic real-time Fourier-type spectral waveform analysis device with or without producing interpretive data; a real-time electrocardiographic interpretive arrhythmia waveform analysis device; a real-time interpretive electrocardiographic ischemic waveform analysis device; a noninvasive plethysmographic or doppler peripheral pulse waveform data gathering device; invasive waveform and/or pressure data means gathering data from the systemic arterial system, pulmonary arterial system, occluded pulmonary arterial system, and cardiac chambers; a device for providing continuous thermodilution cardiac output data from invasive catheter(s); means for providing continuous mixed venous oxygen hemoglobin saturation data; means for supplying echocardiographic data in one, two, or three dimensions.

After a period of training and testing of the resulting model, the system is run without input from event organizer 6, and brain monitor 1. The resulting trained system 3, depicted in FIG. 1b, where only cardiovascular data 5 is provided via cardiovascular monitoring means 4; makes a prediction via 16 to virtual neuro monitor 17 that simulates the situation as if actual brain monitoring were in place. This apparent but absent "virtual" brain monitor 19 input 20 is depicted with stippled lines and represents the virtual input that produces output 16 by way of cardiovascular monitor means 4 passing data 5 to computer classification and prediction means 3, now trained to predict the output that would appear on display means 17 as if real brain monitor means 1 were present. This ability of computer classification and prediction means 3 to make such predictions is based on the training provided as was discussed with reference to FIG. 1a.

Since some of the data manipulation required for adequate training of computer classification and prediction means 3 may require considerably more time than allowable during real-time processing, FIG. 2 depicts a method by which real-time data is stored in input data storage means 21 and given a time label by timer means 22. Event organizer 6 can provide input to data storage means 21 or provide input directly into computer classification and prediction means 3. Timer means 22 also allows release of stored brain, cardiovascular, and event data to computer means 3 for training and training manipulation in a mode slower than real time. This method is particularly useful for allowing human operator input 11 to act on otherwise normally rapidly acquired input data.

In situations where it is feasible to use brain monitoring means 1 in real-time for actual subject monitoring even when computer classification and prediction means 3 is already trained to make predictions from cardiovascular data input 5 as described via FIG. 1a, FIG. 1b, and FIG. 2; the computer means 3 can act as an aid to detect and replace artifact that would otherwise interfere with adequate interpretation of a signal based on brain monitor data 5 alone.

In the artifact detection and replacement means outlined in block diagram in FIG. 3, computer classification and prediction means 3 is already in a trained state to act as a

"virtual" brain monitor by making predictions solely based on cardiovascular data 5. This predicted "virtual" brain data is transferred via 16 to comparators 23 and 27. In comparator means 23, "virtual" brain data derived from cardiovascular data 5 via trained computer means 3 is combined with actual brain data 2 coming from brain monitor means 1. Comparator means 23 specifically compares the signals and the resulting signal 24 comprises a flat signal interspersed with pieces labeled as artifact that were identified by the comparator means based on identifying signal patterns from real brain input data 2, that in their actuality, differ significantly from the predicted signal patterns 16.

The artifact signal 24 is then fed to comparator means 25 and compared to actual brain monitor signal 2, said comparator means 25 designed to splice real signal with artifact areas deleted, resulting in signal 26 which comprises a real signal reproduction with gaps where artifact has been identified.

The real brain monitor signal with gaps 26 is fed to comparator means 27 which fills these gaps with virtual brain monitor signal 16 predicted by computer means 3 from cardiovascular input 5. The result is spliced signal 28 comprising real brain data signal 2 with areas of identified artifact replaced by "virtual" predicted data signal 16. This resulting signal is fed to display means 17 for use by the operator.

In the preferred embodiment, the cardiovascular monitoring means 4 comprises a combination of electrocardiographic, bioimpedance, and pulse plethysmographic data as depicted in FIG. 4a. In this version of the invention, 3-4 or more electrocardiographic monitoring leads 29 (1,2,3, . . . N) in addition to supplying data to electrocardiographic monitor means 33 and to computer means 3 via 34, are also used to obtain data on thoracic bioimpedance. Here, low pass filter 30 allows ECG signals to pass but prevents any input/intereference by higher frequency bioimpedance signals. These signals are conveyed via 3-4 or more electrical conduits 31 (1,2,3, . . . N) to bioimpedance apparatus 35 which in combination with data from pulse plethysmograph 36 data 37 to pulse plethysmograph signal processor 38, benefits from signal 39 which provides an additional estimate of systolic ejection time in accordance with the Giuffre Anzano method described. The resulting bioimpedance cardiovascular parameter data 46 produced are transferred to computing means 3. Bioimpedance means 35 also comprises an optimization means to detect and determine the optimal combination of leads 31 (1,2,3, . . . N) to input said bioimpedance signal and detect it from various input and output lead 29 arrangements.

The above referenced Giuffre Anzano method by which pulse plethysmographic data 39 can assist bioimpedance apparatus 35 in producing more accurate cardiovascular parameter data as described is outlined briefly in FIG. 4b. Plethysmographic pulse signal 37 is fed to plethysmograph signal processor 38 where major deflections from baseline 41 from plethysmographic waveform 41 are detected and measured, producing systolic ejection time estimate 40. Estimate 40 is sent as data 39 to multiplier 45 within bioimpedance apparatus 35 which is fed bioimpedance data from 3-4 or more leads 1,2,3, . . . N via signal processor and differentiation means 42 also located within bioimpedance means 35. Two resulting signals include baseline thoracic impedance 43 and maximum rate of change in bioimpedance 44. These signals multiplied produce cardiovascular data estimate 46, fed to computer means 3.

The above description of the diagrams and the preferred embodiment are one possible method of carrying out the

purposes outlined herein and as a means for explaining some of the principles claimed. The various features of novelty which characterize the invention are pointed out with particularity in the claims annexed to and forming a part of this disclosure.

While specific embodiments of the invention have been shown and described in detail to illustrate the application of the principles of the invention, it will be understood that the invention may be embodied otherwise without departing from such principles.

What is claimed is:

1. A monitoring system, comprising:
 a cardiovascular monitoring means for providing cardiovascular data;
 a brain monitoring means for providing brain monitoring data;
 classification and prediction computing means for processing said cardiovascular data in a real-time fashion, said computing means comprising a trainable system having a training phase comprising analysis of said cardiovascular data said brain monitoring data collected from a same patient or precorrelated data, to provide a trained state, said classification and prediction computing means, when in said trained state providing brain monitoring prediction data on the basis of said cardiovascular monitoring data without the use of said brain monitoring data.

2. The monitoring system in accordance with claim 1, wherein said computing means training phase may occur at a slower rate than a real time processing rate to allow for input from one of a human operator and additional computer analytical tools to optimize said training phase.

3. The monitoring system in accordance with claim 1, further comprising event data means for providing event data, wherein said computing means training phase further includes analysis of said event data with said analysis of said cardiovascular data said brain monitoring data.

4. The monitoring system in accordance with claim 1, wherein said computing means in said trained state feeds back to a said brain monitoring means to improve noise artifact recognition by comparing predicted brain monitor output data to actual brain monitor output data.

5. The monitoring system in accordance with claim 3, wherein said event data means includes one or more of:
 an expired gas concentration evaluation system for obtaining expired gas concentration data;
 a drug infusion device providing drug infusion data;
 input gas concentration regulation means providing input gas concentration data;
 means for supplying data derived from spreadsheet type data depositories;
 human operator input based on subjective and/or objective evaluation of the subject being monitored;
 expired ethanol analysis;
 magnesium levels monitoring means for obtaining magnesium therapy monitoring data;
 specific drug or alcohol blood level monitoring means to provide drug/alcohol blood level data;
 a standard skin galvanic resistance lie detector for providing galvanic lie detector resistance data;
 a meditation state source data indicating achieved states of meditation;
 means for monitoring specific events during surgery and providing surgery event data;

g-force measurement means for providing g-force data within an aircraft, spacecraft, and/or flight simulator; and

means for providing psychometric performance data based on answers to questions and/or performance of specific tasks.

6. The monitoring system in accordance with claim 1, wherein said training phase can continue beyond an initial training period wherein said brain monitoring prediction data is provided on the basis of said cardiovascular monitoring data without the use of said brain monitoring data after said initial training period.

7. The monitoring system in accordance with claim 1, wherein said cardiovascular monitoring means comprises one or more of:

a thoracic bioimpedance monitoring means;

a modified thoracic bioimpedance monitoring means that concomitantly utilizes electrodes positioned on the subject and used for electrocardiographic analysis;

a heart rate variability real-time series analysis device; a electrocardiographic morphology analysis device operating in real time and producing interpretive data;

a electrocardiographic real-time Fourier-type spectral waveform analysis device with or without producing interpretive data;

a real-time electrocardiographic interpretive arrhythmia waveform analysis device;

a real-time interpretive electrocardiographic ischemic waveform analysis device;

a noninvasive plethysmographic or doppler peripheral pulse waveform data gathering device;

invasive waveform and/or pressure data means gathering data from the systemic arterial system, pulmonary arterial system, occluded pulmonary arterial system, and cardiac chambers;

a device for providing continuous thermodilution cardiac output data from invasive catheter(s);

means for providing continuous mixed venous oxygen hemoglobin saturation data; and

means for supplying echocardiographic data in one, two, or three dimensions.

8. The monitoring system in accordance with claim 1, wherein said brain monitoring means comprises one or more of:

a full multiple lead electromyography device; full multiple lead electroencephalography device with individual Fourier wave analysis in real time of every channel;

a processed EEG resulting in data comprising complex graphical or numeric output, simplified graphical or numeric output, and/or a simple scaled numerical value correlating with general cerebral unilateral or bilateral cortical activity;

an evoked potential measurement means from somatosensory evoked potentials, brainstem auditory evoked potentials, visual evoked potentials, and/or motor evoked potentials;

a positron emission tomography device; a dynamic magnetic resonance imaging device; and a facial electromyography device.

9. The monitoring system in accordance with claim 1, wherein an output is set to provide a warning to the operator when the subject achieves one or more preset threshold levels of predicted brain monitor output.

10. The monitoring system in accordance with claim 1, wherein an output is coupled to an operator warning signal when the subject reaches critical levels consistent with reduced brain bloodflow.

11. The monitoring system in accordance with claim 1, wherein an is set to provide an indication of seizure activity in a pharmacologically paralyzed subject as would occur during psychiatric electroconvulsive therapy.

12. A patient monitoring process, comprising the steps of:

providing cardiovascular data;

providing brain monitoring data;

providing a computing device training phase by providing a trainable computer system receiving said cardiovascular data and said brain monitoring data during a training phase including analysis of said cardiovascular data said brain monitoring data collected from a same patient or precorrelated data to provide a trained state or partially trained state;

providing additional cardiovascular data; and

processing said additional cardiovascular data in a real-time fashion in said trained state or partially trained state to provide brain monitoring prediction data on the basis of said additional cardiovascular monitoring data without the use of said brain monitoring data and said cardiovascular data.

13. The monitoring process in accordance with claim 12, wherein said computing means training phase may occur at a slower rate than a real time processing rate to allow for input from one of a human operator and additional computer analytical tools to optimize said training phase.

14. The monitoring process in accordance with claim 12, further comprising providing event data means for providing event data, wherein said computing means training phase further includes analysis of said event data with said analysis of said cardiovascular data said brain monitoring data.

15. The monitoring process in accordance with claim 12, wherein said computing means in said trained state feeds back to a said brain monitoring means to improve noise artifact recognition by comparing predicted brain monitor output data to actual brain monitor output data.

16. The monitoring process in accordance with claim 14, wherein said event data means includes one or more of:

an expired gas concentration evaluation process for obtaining expired gas concentration data;

a drug infusion device providing drug infusion data; input gas concentration regulation means providing input gas concentration data;

means for supplying data derived from spreadsheet type data depositories;

human operator input based on subjective and/or objective evaluation of the subject being monitored;

expired ethanol analysis;

magnesium levels monitoring means for obtaining magnesium therapy monitoring data;

specific drug or alcohol blood level monitoring means to provide drug/alcohol blood level data;

a standard skin galvanic resistance fz detector for providing galvanic fz detector resistance data;

a meditation state source data indicating achieved states of meditation;

means for monitoring specific events during surgery and providing surgery event data;

g-force measurement means for providing g-force data within an aircraft, spacecraft, and/or flight simulator; and

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means for providing psychometric performance data based on answers to questions and(or) performance of specific tasks.

17. The monitoring process in accordance with claim 12, wherein said training phase can continue beyond an initial training period wherein said brain monitoring prediction data is provided on the basis of said cardiovascular monitoring data without the use of said brain monitoring data after said initial training period.

18. The monitoring process in accordance with claim 12, wherein said cardiovascular monitoring data is obtained from one or more of:

- a thoracic bioimpedance monitoring means;
- a modified thoracic bioimpedance monitoring means that concomitantly utilizes electrodes positioned on the subject and used for electrocardiographic analysis;
- a heart rate variability real-time series analysis device;
- a echocardiographic morphology analysis device operating in real time and producing interpretive data;
- a electrocardiographic real-time Fourier-type spectral waveform analysis device with or without producing interpretive data;
- a real-time electrocardiographic interpretive arrhythmia waveform analysis device;
- a real-time interpretive electrocardiographic ischemic waveform analysis device;
- a noninvasive piezysmographic or doppler peripheral pulse waveform data gathering device;
- invasive waveform and(or) pressure data means gathering data from the systemic arterial system, pulmonary arterial system, occluded pulmonary arterial system, and cardiac chambers;

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a device for providing continuous thermodilution cardiac output data from invasive catheter(s);

means for providing continuous mixed venous oxygen hemoglobin saturation data; and

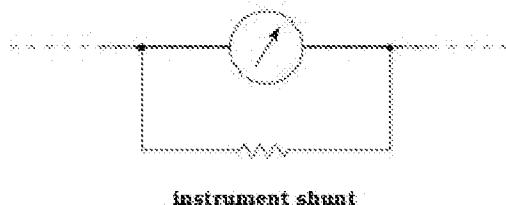
means for supplying echocardiographic data in one, two, or three dimensions.

19. The monitoring process in accordance with claim 12, wherein said brain monitoring data is obtained from one or more of:

- a full multiple lead electroencephalography device;
- a full multiple lead electroencephalography device with individual Fourier wave analysis in real time of every channel;
- a processed EEG resulting in data comprising complex graphical or numeric output, simplified graphical or numeric output, and(or) a simple scaled numerical value correlating with general cerebral unilateral or bilateral cortical activity;
- an evoked potential measurement means from somatosensory evoked potentials, brainstem auditory evoked potentials, visual evoked potentials, and(or) motor evoked potentials;
- a positron emission tomography device;
- a dynamic magnetic resonance imaging device; and
- a facial electromyography device.

20. The monitoring process in accordance with claim 12, wherein an output is set to provide a warning to the operator when the subject achieves one or more preset threshold levels of predicted brain monitor output.

* * * * *



For ammeters, it is called a current transformer; for voltmeters, it is called a potential transformer.

insulant A nonconducting material, used to prevent the flow of electric current between or among points. See INSULATOR, 1.

insulated Isolated from conductors by an INSULANT.

insulated-gate field-effect transistor Abbreviation, IGBT. See METAL-OXIDE SILICON FET.

insulated resistor A resistor around which is molded a nonconducting material, such as vitreous enamel or a plastic.

insulating tape Electrical insulation in the form of a thin, usually adhesive, strip of fabric, paper, or plastic.

insulation 1. A coating of dielectric material that prevents a short circuit between a conductor and the surrounding environment. 2. The application of a dielectric coating to an electrical conductor. 3. Electrical separation between or among different components, circuits, or systems.

insulation breakdown Current leakage through, and rupture of, an insulating material because of high-voltage stress.

insulation ratings Collectively, the dielectric constant, dielectric strength, power factor, and resistivity of an insulating material. Sometimes included are such physical properties as rupture strength, melting point, etc.

insulation resistance The very high resistance exhibited by a good insulating material. It is expressed in megohms (or higher units of resistance) for a sample of material of stated volume or area.

insulation system Collectively, the materials needed to insulate a given electronic device.

insulator 1. A material that, ideally, conducts no electricity; it can, therefore, be used for insulation and protection of energized circuits and components (also see DIELECTRIC). Actually, no insulator is perfectly nonconductive (see, for example, INSULATION RESISTANCE). 2. A molded piece of solid insulating material, used to electrically insulate conductors—especially in antenna systems and power transmission lines. 3. Any body made from an insulating material.

insulator arcover A sudden arc, or flow of current, over the surface of an insulator, because of excessive voltage.

integer A positive or negative whole number, as opposed to a fraction or mixed number.

integral 1. Also called indefinite integral and antiderivative. For given mathematical function f , function g , whose derivative is equal to f . 2. Also called definite integral. The area under a curve of a function, between two vertical lines defined by two specific points in the domain of the function. 3. The part of a number to the left of the radix point. 4. Pertaining to integers (positive or negative whole numbers) or quantities that can be represented by integers.

integral action In automatic control operations, a control action delivering a corrective signal proportional to the time that the controlled quantity has differed from a desired value.

integral contact In a relay or switch, a contact that carries current to be switched.

integral-horsepower motor A motor rated at one horsepower.

integral multiple A whole multiple of a number. Thus, a harmonic is an integral multiple of fundamental frequency f , $2f$, $3f$, $4f$, etc.

integral number See INTEGER.

integrate 1. To perform the function of mathematical or electrical INTEGRATION. 2. To construct a circuit on a piece of semiconductor material.

integrated Constructed on a single piece of material, such as a semiconductor wafer.

integrated amplifier An audio-frequency (AF) amplifier having a preamplifier, intermediate amplifier, and output amplifier on a single chassis.

integrated capacitor In an integrated circuit, a fixed capacitor in which one plate is a layer of material diffused into the substrate, the dielectric is a thin-oxide film grown on top of the first layer, and the other plate is a metal layer deposited on top of the oxide film.

integrated circuit Abbreviation, IC. A circuit whose components and connecting "wires" are made by processing distinct areas of a chip of semiconductor material, such as silicon. Classified according to construction (e.g., monolithic IC, thin-film IC, hybrid IC).

integrated data processing Abbreviation, IDP. The detailed electronic classification, sorting, storage, and mathematical processing of data within a coordinated system of equipment, usually at one location.

integrated electronics The branch of electronics that is concerned with the design and fabrication of integrated circuits.

integrated resistor See DIFFUSED-LAYER RESISTOR.

Integrated Services Digital Network Abbreviation, ISDN. A communications network or connection intended primarily for Internet access via telephone lines. Allows significantly higher data speed than is possible with a conventional analog connection. In addition, it is possible to use a digital system, such as a computer, online simultaneously with an analog voice conversation.

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2143 >Examples of< Basic Requirements of a - 2100 Patentability

2143 >Examples of< Basic Requirements of a *Prima Facie* Case of Obviousness

**>The Supreme Court in *KSR International Co. v. Teleflex Inc.*, 550 U.S. ___, ___, 82 USPQ2d 1385, 1395-97 (2007) identified a number of rationales to support a conclusion of obviousness which are consistent with the proper "functional approach" to the determination of obviousness as laid down in *Graham*. The key to supporting any rejection under 35 U.S.C. 103 is the clear articulation of the reason(s) why the claimed invention would have been obvious. The Supreme Court in *KSR* noted that the analysis supporting a rejection under 35 U.S.C. 103 should be made explicit.

EXEMPLARY RATIONALES

Exemplary rationales that may support a conclusion of obviousness include:

- (A) Combining prior art elements according to known methods to yield predictable results;
- (B) Simple substitution of one known element for another to obtain predictable results;
- (C) Use of known technique to improve similar devices (methods, or products) in the same way;
- (D) Applying a known technique to a known device (method, or product) ready for improvement to yield predictable results;
- (E) "Obvious to try" - choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success;
- (F) Known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the art;
- (G) Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.

Note that the list of rationales provided is not intended to be an all-inclusive list. Other rationales to support a conclusion of obviousness may be relied upon by Office personnel.

The subsections below include discussions of each rationale along with examples illustrating how the cited rationales may be used to support a finding of obviousness. The cases cited (from which the facts were derived) may not necessarily stand for the proposition that the particular rationale is the basis for the court's holding of obviousness. Note that, in some instances, a single case is used in different subsections to illustrate the use of more than one rationale to support a finding of obviousness. It will often be the case that, once the *Graham* inquiries have been satisfactorily resolved, a conclusion of obviousness may be supported by more than one line of reasoning.

A. Combining Prior Art Elements According to Known Methods To Yield Predictable Results

To reject a claim based on this rationale, Office personnel must resolve the *Graham* factual inquiries. Then, Office personnel must articulate the following:

- (1) a finding that the prior art included each element claimed, although not necessarily in a single prior art reference, with the only difference between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference;
- (2) a finding that one of ordinary skill in the art could have combined the elements as claimed by known methods, and that in combination, each element merely performs the same function as it does separately;
- (3) a finding that one of ordinary skill in the art would have recognized that the results of the combination were predictable; and
- (4) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

The rationale to support a conclusion that the claim would have been obvious is that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination yielded nothing more than predictable results to one of ordinary skill in the art. *KSR*, 550 U.S. at ___, 82 USPQ2d at 1395; *Sakraida v. AG Pro, Inc.*, 425 U.S. 273, 282, 189 USPQ 449, 453 (1976); *Anderson's-Black Rock, Inc. v. Pavement Salvage Co.*, 396 U.S. 57, 62-63, 163 USPQ 673, 675 (1969); *Great Atlantic & P. Tea Co. v. Supermarket Equipment Corp.*, 340 U.S. 147, 152, 87 USPQ 303, 306 (1950). "[I]t can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does." *KSR*, 550 U.S. at ___, 82 USPQ2d at 1396. If any of these findings cannot be made, then this rationale cannot be used to support a conclusion that the claim would have been obvious to one of ordinary skill in the art.

Example 1:

The claimed invention in *Anderson's-Black Rock, Inc. v. Pavement Salvage Co.*, 396 U.S.

57, 163 USPQ 673 (1969) was a paving machine which combined several well-known elements onto a single chassis. Standard prior art paving machines typically combined equipment for spreading and shaping asphalt onto a single chassis. The patent claim included the well-known element of a radiant-heat burner attached to the side of the paver for the purpose of preventing cold joints during continuous strip paving. The prior art used radiant heat for softening the asphalt to make patches, but did not use radiant heat burners to achieve continuous strip paving. All of the component parts were known in the prior art. The only difference was the combination of the "old elements" into a single device by mounting them on a single chassis. The Court found that the operation of the heater was in no way dependent on the operation of the other equipment, and that a separate heater could also be used in conjunction with a standard paving machine to achieve the same results. The Court concluded that "[t]he convenience of putting the burner together with the other elements in one machine, though perhaps a matter of great convenience, did not produce a 'new' or 'different function'" and that to those skilled in the art the use of the old elements in combination would have been obvious. *Id.* at 60, 163 USPQ at 674.

Note that combining known prior art elements is not sufficient to render the claimed invention obvious if the results would not have been predictable to one of ordinary skill in the art. *United States v. Adams*, 383 U.S. 39, 51-52, 148 USPQ 479, 483-84 (1966). In *Adams*, the claimed invention was to a battery with one magnesium electrode and one cuprous chloride electrode that could be stored dry and activated by the addition of plain water or salt water. Although magnesium and cuprous chloride were individually known battery components, the Court concluded that the claimed battery was nonobvious. The Court stated that "[d]espite the fact that each of the elements of the Adams battery was well known in the prior art, to combine them as did Adams required that a person reasonably skilled in the prior art must ignore" the teaching away of the prior art that such batteries were impractical and that water-activated batteries were successful only when combined with electrolytes detrimental to the use of magnesium electrodes. *Id.* at 42-43, 50-52, 148 USPQ at 480, 483. "When the prior art teaches away from combining certain known elements, discovery of successful means of combining them is more likely to be nonobvious." *KSR*, 550 U.S. at ___, 82 USPQ2d at 1395.

Example 2:

The claimed invention in *Ruiz v. AB Chance Co.*, 357 F.3d 1270, 69 USPQ2d 1686 (Fed. Cir. 2004) was directed to a system which employs a screw anchor for underpinning existing foundations and a metal bracket to transfer the building load onto the screw anchor. The prior art (Fuller) used screw anchors for underpinning existing structural foundations. Fuller used a concrete haunch to transfer the load of the foundation to the screw anchor. The prior art (Gregory) used a push pier for underpinning existing structural foundations. Gregory taught a method of transferring load using a bracket, specifically: a metal bracket transfers the foundation load to the push pier. The pier is driven into the ground to support the load. Neither reference showed the two elements of the claimed invention - screw anchor and metal bracket - used together. The court found that "artisans knew that a foundation underpinning system requires a means of connecting the foundation to the load-bearing member." *Id.* at 1276, 69 USPQ2d at 1691.

The nature of the problem to be solved - underpinning unstable foundations - as well as the need to connect the member to the foundation to accomplish this goal, would have led one of ordinary skill in the art to choose an appropriate load bearing member and a

compatible attachment. Therefore, it would have been obvious to use a metal bracket (as shown in Gregory) in combination with the screw anchor (as shown in Fuller) to underpin unstable foundations.

B. Simple Substitution of One Known Element for Another To Obtain Predictable Results

To reject a claim based on this rationale, Office personnel must resolve the *Graham* factual inquiries. Then, Office personnel must articulate the following:

- (1) a finding that the prior art contained a device (method, product, etc.) which differed from the claimed device by the substitution of some components (step, element, etc.) with other components;
- (2) a finding that the substituted components and their functions were known in the art;
- (3) a finding that one of ordinary skill in the art could have substituted one known element for another, and the results of the substitution would have been predictable; and
- (4) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

The rationale to support a conclusion that the claim would have been obvious is that the substitution of one known element for another yields predictable results to one of ordinary skill in the art. If any of these findings cannot be made, then this rationale cannot be used to support a conclusion that the claim would have been obvious to one of ordinary skill in the art.

Example 1:

The claimed invention in *In re Fout*, 675 F.2d 297, 213 USPQ 532 (CCPA 1982) was directed to a method for decaffeinating coffee or tea. The prior art (Pagliaro) method produced a decaffeinated vegetable material and trapped the caffeine in a fatty material (such as oil). The caffeine was then removed from the fatty material by an aqueous extraction process. Applicant (Fout) substituted an evaporative distillation step for the aqueous extraction step. The prior art (Waterman) suspended coffee in oil and then directly distilled the caffeine through the oil. The court found that "[b]ecause both Pagliaro and Waterman teach a method for separating caffeine from oil, it would have been *prima facie* obvious to substitute one method for the other. Express suggestion to substitute one equivalent for another need not be present to render such substitution obvious." *Id.* at 301, 213 USPQ at 536.

Example 2:

The invention in *In re O'Farrell*, 853 F.2d 894, 7 USPQ2d 1673 (Fed. Cir. 1988) was directed to a method for synthesizing a protein in a transformed bacterial host species by substituting a heterologous gene for a gene native to the host species. Generally speaking, protein synthesis *in vivo* followed the path of DNA to RNA to protein. Although the prior art Polisky article (authored by two of the three inventors of the application) had explicitly suggested employing the method described for protein synthesis, the inserted heterologous gene exemplified in the article was one that normally did not proceed all the

way to the protein production step, but instead terminated with the RNA. A second reference to Bahl had described a general method of inserting chemically synthesized DNA into a plasmid. Thus, it would have been obvious to one of ordinary skill in the art to replace the prior art gene with another gene known to lead to protein production, because one of ordinary skill in the art would have been able to carry out such a substitution, and the results were reasonably predictable.

In response to applicant's argument that there had been significant unpredictability in the field of molecular biology at the time of the invention, the court stated that the level of skill was quite high and that the teachings of Polisky, even taken alone, contained detailed enabling methodology and included the suggestion that the modification would be successful for synthesis of proteins.

This is not a situation where the rejection is a statement that it would have been "obvious to try" without more. Here there was a reasonable expectation of success. "Obviousness does not require absolute predictability of success." *Id.* at 903, 7 USPQ2d at 1681.

Example 3:

The fact pattern in *Ruiz v. AB Chance Co.*, 357 F.3d 1270, 69 USPQ2d 1686 (Fed. Cir. 2004) is set forth above in Example 2 in subsection A.

The prior art showed differing load-bearing members and differing means of attaching the foundation to the member. Therefore, it would have been obvious to one of ordinary skill in the art to substitute the metal bracket taught in Gregory for Fuller's concrete haunch for the predictable result of transferring the load.

Example 4:

The claimed invention in *Ex parte Smith*, 83 USPQ2d 1509 (Bd. Pat. App. & Int. 2007), was a pocket insert for a bound book made by gluing a base sheet and a pocket sheet of paper together to form a continuous two-ply seam defining a closed pocket. The prior art (Wyant) disclosed at least one pocket formed by folding a single sheet and securing the folder portions along the inside margins using any convenient bonding method. The prior art (Wyant) did not disclose bonding the sheets to form a continuous two-ply seam. The prior art (Dick) disclosed a pocket that is made by stitching or otherwise securing two sheets along three of its four edges to define a closed pocket with an opening along its fourth edge.

In considering the teachings of Wyant and Dick, the Board "found that (1) each of the claimed elements is found within the scope and content of the prior art; (2) one of ordinary skill in the art could have combined the elements as claimed by methods known at the time the invention was made; and (3) one of ordinary skill in the art would have recognized at the time the invention was made that the capabilities or functions of the combination were predictable." Citing KSR, the Board concluded that "[t]he substitution of the continuous, two-ply seam of Dick for the folded seam of Wyant thus is no more than the simple substitution of one known element for another or the mere application of a known technique to a piece of prior art ready for improvement.

C. Use of Known Technique To Improve Similar Devices (Methods, or Products) in the Same Way

To reject a claim based on this rationale, Office personnel must resolve the *Graham* factual inquiries. Then, Office personnel must articulate the following:

- (1) a finding that the prior art contained a "base" device (method, or product) upon which the claimed invention can be seen as an "improvement;"
- (2) a finding that the prior art contained a "comparable" device (method, or product that is not the same as the base device) that has been improved in the same way as the claimed invention;
- (3) a finding that one of ordinary skill in the art could have applied the known "improvement" technique in the same way to the "base" device (method, or product) and the results would have been predictable to one of ordinary skill in the art; and
- (4) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

The rationale to support a conclusion that the claim would have been obvious is that a method of enhancing a particular class of devices (methods, or products) has been made part of the ordinary capabilities of one skilled in the art based upon the teaching of such improvement in other situations. One of ordinary skill in the art would have been capable of applying this known method of enhancement to a "base" device (method, or product) in the prior art and the results would have been predictable to one of ordinary skill in the art. The Supreme Court in *KSR* noted that if the actual application of the technique would have been beyond the skill of one of ordinary skill in the art, then using the technique would not have been obvious. *KSR*, 550 U.S. at ___, 82 USPQ2d at 1396. If any of these findings cannot be made, then this rationale cannot be used to support a conclusion that the claim would have been obvious to one of ordinary skill in the art.

Example 1:

The claimed invention in *In re Nilssen*, 851 F.2d 1401, 7 USPQ2d 1500 (Fed. Cir. 1988) was directed to a "means by which the self-oscillating inverter in a power-line-operated inverter-type fluorescent lamp ballast is disabled in case the output current from the inverter exceeds some pre-established threshold level for more than a very brief period." *Id.* at 1402, 7 USPQ2d at 1501 That is, the current output was monitored, and if the current output exceeded some threshold for a specified short time, an actuation signal was sent and the inverter was disabled to protect it from damage.

The prior art (a USSR certificate) described a device for protecting an inverter circuit in an undisclosed manner via a control means. The device indicated the high-load condition by way of the control means, but did not indicate the specific manner of overload protection. The prior art (Kammiller) disclosed disabling the inverter in the event of a high-load current condition in order to protect the inverter circuit. That is, the overload protection was achieved by disabling the inverter by means of a cutoff switch.

The court found "it would have been obvious to one of ordinary skill in the art to use the threshold signal produced in the USSR device to actuate a cutoff switch to render the inverter inoperative as taught by Kammiller." *Id.* at 1403, 7 USPQ2d at 1502. That is, using the known technique of a cutoff switch for protecting a circuit to provide the protection desired in the inverter circuit of the USSR document would have been obvious

to one of ordinary skill.

Example 2:

The fact pattern in *Ruiz v. AB Chance Co.* 357 F.3d 1270, 69 USPQ2d 1686 (Fed. Cir. 2004) is set forth above in Example 2 in subsection A.

The nature of the problem to be solved may lead inventors to look at references relating to possible solutions to that problem. *Id.* at 1277, 69 USPQ2d at 1691. Therefore, it would have been obvious to use a metal bracket (as shown in Gregory) with the screw anchor (as shown in Fuller) to underpin unstable foundations.

D. Applying a Known Technique to a Known Device (Method, or Product) Ready for Improvement To Yield Predictable Results

To reject a claim based on this rationale, Office personnel must resolve the *Graham* factual inquiries. Then, Office personnel must articulate the following:

- (1) a finding that the prior art contained a "base" device (method, or product) upon which the claimed invention can be seen as an "improvement;"
- (2) a finding that the prior art contained a known technique that is applicable to the base device (method, or product);
- (3) a finding that one of ordinary skill in the art would have recognized that applying the known technique would have yielded predictable results and resulted in an improved system; and
- (4) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

The rationale to support a conclusion that the claim would have been obvious is that a particular known technique was recognized as part of the ordinary capabilities of one skilled in the art. One of ordinary skill in the art would have been capable of applying this known technique to a known device (method, or product) that was ready for improvement and the results would have been predictable to one of ordinary skill in the art. If any of these findings cannot be made, then this rationale cannot be used to support a conclusion that the claim would have been obvious to one of ordinary skill in the art.

Example 1:

The claimed invention in *Dann v. Johnston*, 425 U.S. 219, 189 USPQ 257 (1976) was directed towards a system (i.e., computer) for automatic record keeping of bank checks and deposits. In this system, a customer would put a numerical category code on each check or deposit slip. The check processing system would record these on the check in magnetic ink, just as it does for amount and account information. With this system in place, the bank can provide statements to customers that are broken down to give subtotals for each category. The claimed system also allowed the bank to print reports according to a style requested by the customer. As characterized by the Court, "[u]nder respondent's invention, then, a general purpose computer is programmed to provide bank customers with an individualized and categorized breakdown of their transactions

during the period in question." *Id.* at 222, 189 USPQ at 259.

BASE SYSTEM - The nature of the use of data processing equipment and computer software in the banking industry was that banks routinely did much of the record-keeping automatically. In routine check processing, the system read any magnetic ink characters identifying the account and routing. The system also read the amount of the check and then printed that value in a designated area of the check. The check was then sent through a further data processing step which used the magnetic ink information to generate the appropriate records for transactions and for posting to the appropriate accounts. These systems included generating periodic statements for each account, such as the monthly statement sent to checking account customers.

IMPROVED SYSTEM - The claimed invention supplemented this system by recording a category code which can then be utilized to track expenditures by category. Again, the category code will be a number recorded on the check (or deposit slip) which will be read, converted into a magnetic ink imprint, and then processed in the data system to include the category code. This enabled reporting of data by category as opposed to only allowing reporting by account number.

KNOWN TECHNIQUE - This is an application of a technique from the prior art - the use of account numbers (generally used to track an individual's total transactions) to solve the problem of how to track categories of expenditures to more finely account for a budget. That is, account numbers (identifying data capable of processing in the automatic data processing system) were used to distinguish between different customers. Furthermore, banks have long segregated debits attributable to service charges within any given separate account and have rendered their customers subtotals for those charges. Previously, one would have needed to set up separate accounts for each category and thus receive separate reports. Supplementing the account information with additional digits (the category codes) solved the problem by effectively creating a single account that can be treated as distinct accounts for tracking and reporting services. That is, the category code merely allowed what might previously have been separate accounts to be handled as a single account, but with a number of sub-accounts indicated in the report.

The basic technique of putting indicia on data which then enabled standard sorting, searching, and reporting yielded no more than the predictable outcome which one of ordinary skill would have expected to achieve with this common tool of the trade and was therefore an obvious expedient. The Court held that "[t]he gap between the prior art and respondent's system is simply not so great as to render the system nonobvious to one reasonably skilled in the art." *Id.* at 230, 189 USPQ at 261.

Example 2:

The fact pattern in *In re Nilssen*, 851 F.2d 1401, 7 USPQ2d 1500 (Fed. Cir. 1988) is set forth above in Example 1 in subsection C.

The court found "it would have been obvious to one of ordinary skill in the art to use the threshold signal produced in the USSR device to actuate a cutoff switch to render the inverter inoperative as taught by Kammiller." *Id.* at 1403, 7 USPQ2d at 1502. The known technique of using a cutoff switch would have predictably resulted in protecting the inverter circuit. Therefore, it would have been within the skill of the ordinary artisan to use

a cutoff switch in response to the actuation signal to protect the inverter.

E. "Obvious To Try" - Choosing From a Finite Number of Identified, Predictable Solutions, With a Reasonable Expectation of Success

To reject a claim based on this rationale, Office personnel must resolve the *Graham* factual inquiries. Then, Office personnel must articulate the following:

- (1) a finding that at the time of the invention, there had been a recognized problem or need in the art, which may include a design need or market pressure to solve a problem;
- (2) a finding that there had been a finite number of identified, predictable potential solutions to the recognized need or problem;
- (3) a finding that one of ordinary skill in the art could have pursued the known potential solutions with a reasonable expectation of success; and
- (4) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

The rationale to support a conclusion that the claim would have been obvious is that "a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely that product [was] not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show that it was obvious under § 103." *KSR*, 550 U.S. at ___, 82 USPQ2d at 1397. If any of these findings cannot be made, then this rationale cannot be used to support a conclusion that the claim would have been obvious to one of ordinary skill in the art.

Example 1:

The claimed invention in *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 82 USPQ2d 1321 (Fed. Cir. 2007) was directed to the amlodipine besylate drug product, which is commercially sold in tablet form in the United States under the trademark Norvasc®. At the time of the invention, amlodipine was known as was the use of besylate anions. Amlodipine was known to have the same therapeutic properties as were being claimed for the amlodipine besylate but Pfizer discovered that the besylate form had better manufacturing properties (e.g., reduced "stickiness").

Pfizer argued that the results of forming amlodipine besylate would have been unpredictable and therefore nonobvious. The court rejected the notion that unpredictability could be equated with nonobviousness here, because there were only a finite number (53) of **pharmaceutically acceptable** salts to be tested for improved properties.

The court found that one of ordinary skill in the art having problems with the machinability of amlodipine would have looked to forming a salt of the compound and would have been able to narrow the group of potential salt-formers to a group of 53 anions known to form pharmaceutically acceptable salts, which would be an acceptable number to form "a reasonable expectation of success."

Example 2:

The claimed invention in *Alza Corp. v. Mylan Laboratories, Inc.*, 464 F.3d 1286, 80 USPQ2d 1001 (Fed. Cir. 2006) was drawn to sustained-release formulations of the drug oxybutynin in which the drug is released at a specified rate over a 24-hour period. Oxybutynin was known to be highly water-soluble, and the specification had pointed out that development of sustained-release formulations of such drugs presented particular problems.

A prior art patent to Morella had taught sustained-release compositions of highly water-soluble drugs, as exemplified by a sustained-release formulation of morphine. Morella had also identified oxybutynin as belonging to the class of highly water-soluble drugs. The Baichwal prior art patent had taught a sustained-release formulation of oxybutynin that had a different release rate than the claimed invention. Finally, the Wong prior art patent had taught a generally applicable method for delivery of drugs over a 24-hour period. Although Wong mentioned applicability of the disclosed method to several categories of drugs to which oxybutynin belonged, Wong did not specifically mention its applicability to oxybutynin.

The court found that because the absorption properties of oxybutynin would have been reasonably predictable at the time of the invention, there would have been a reasonable expectation of successful development of a sustained-release formulation of oxybutynin as claimed. The prior art, as evidenced by the specification, had recognized the obstacles to be overcome in development of sustained-release formulations of highly water-soluble drugs, and had suggested a finite number of ways to overcome these obstacles. The claims were obvious because it would have been obvious to try the known methods for formulating sustained-release compositions, with a reasonable expectation of success. The court was not swayed by arguments of a lack of absolute predictability.

Example 3:

The claimed invention in *Ex parte Kubin*, 83 USPQ2d 1410 (Bd. Pat. App. & Int. 2007), was an isolated nucleic acid molecule. The claim stated that the nucleic acid encoded a particular polypeptide. The encoded polypeptide was identified in the claim by its partially specified sequence, and by its ability to bind to a specified protein.

A prior art patent to Valiante taught the polypeptide encoded by the claimed nucleic acid, but did not disclose either the sequence of the polypeptide, or the claimed isolated nucleic acid molecule. However, Valiante did disclose that by employing conventional methods such as those disclosed by a prior art laboratory manual by Sambrook, the sequence of the polypeptide could be determined, and the nucleic acid molecule could be isolated. In view of Valiante's disclosure of the polypeptide, and of routine prior art methods for sequencing the polypeptide and isolating the nucleic acid molecule, the Board found that a person of ordinary skill in the art would have had a reasonable expectation that a nucleic acid molecule within the claimed scope could have been successfully obtained.

Relying on *In re Deuel*, 51 F.3d 1552, 34 USPQ2d 1210 (Fed. Cir. 1995), appellant argued that it was improper for the Office to use the polypeptide of the Valiante patent together with the methods described in Sambrook to reject a claim drawn to a specific nucleic acid molecule without providing a reference showing or suggesting a structurally

similar nucleic acid molecule. Citing *KSR*, the Board stated that "when there is motivation to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to anticipated success, it is likely the product not of innovation but of ordinary skill and common sense." The Board noted that the problem facing those in the art was to isolate a specific nucleic acid, and there were a limited number of methods available to do so. The Board concluded that the skilled artisan would have had reason to try these methods with the reasonable expectation that at least one would be successful. Thus, isolating the specific nucleic acid molecule claimed was "the product not of innovation but of ordinary skill and common sense."

F. Known Work in One Field of Endeavor May Prompt Variations of It for Use in Either the Same Field or a Different One Based on Design Incentives or Other Market Forces if the Variations Are Predictable to One of Ordinary Skill in the Art

To reject a claim based on this rationale, Office personnel must resolve the *Graham* factual inquiries. Then, Office personnel must articulate the following:

- (1) a finding that the scope and content of the prior art, whether in the same field of endeavor as that of the applicant's invention or a different field of endeavor, included a similar or analogous device (method, or product);
- (2) a finding that there were design incentives or market forces which would have prompted adaptation of the known device (method, or product);
- (3) a finding that the differences between the claimed invention and the prior art were encompassed in known variations or in a principle known in the prior art;
- (4) a finding that one of ordinary skill in the art, in view of the identified design incentives or other market forces, could have implemented the claimed variation of the prior art, and the claimed variation would have been predictable to one of ordinary skill in the art; and
- (5) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

The rationale to support a conclusion that the claimed invention would have been obvious is that design incentives or other market forces could have prompted one of ordinary skill in the art to vary the prior art in a predictable manner to result in the claimed invention. If any of these findings cannot be made, then this rationale cannot be used to support a conclusion that the claim would have been obvious to one of ordinary skill in the art.

Example 1:

The fact pattern in *Dann v. Johnston*, 425 U.S. 219, 189 USPQ 257 (1976) is set forth above in Example 1 in subsection D.

The Court found that the problem addressed by applicant - the need to give more detailed breakdown by a category of transactions - was closely analogous to the task of keeping track of the transaction files of individual business units. *Id.* at 229, 189 USPQ at 261. Thus, an artisan in the data processing area would have recognized the similar class of problem and the known solutions of the prior art and it would have been well within the

ordinary skill level to implement the system in the different environment. The Court held that "[t]he gap between the prior art and respondent's system is simply not so great as to render the system nonobvious to one reasonably skilled in the art." *Id.* at 230, 189 USPQ at 261.

Example 2:

The claimed invention in *Leapfrog Enterprises, Inc. v. Fisher-Price, Inc.*, 485 F.3d 1157, 82 USPQ2d 1687 (Fed. Cir. 2007) was directed to a learning device to help young children read phonetically. The claim read as follows:

An interactive learning device, comprising:

a housing including a plurality of switches;

a sound production device in communication with the switches and including a processor and a memory;

at least one depiction of a sequence of letters, each letter being associable with a switch; and

a reader configured to communicate the identity of the depiction to the processor,

wherein selection of a depicted letter activates an associated switch to communicate with the processor, causing the sound production device to generate a signal corresponding to a sound associated with the selected letter, the sound being determined by a position of the letter in the sequence of letter.

The court concluded that the claimed invention would have been obvious in view of the combination of two pieces of prior art, (1) Bevan (which showed an electro-mechanical toy for phonetic learning), (2) the Super Speak & Read device (SSR) (an electronic reading toy), and the knowledge of one of ordinary skill in the art.

The court made clear that there was no technological advance beyond the skill shown in the SSR device. The court stated that "one of ordinary skill in the art of children's learning toys would have found it obvious to combine the Bevan device with the SSR to update it using modern electronic components in order to gain the commonly understood benefits of such adaptation, such as decreased size, increased reliability, simplified operation, and reduced cost. While the SSR only permits generation of a sound corresponding to the first letter of a word, it does so using electronic means. The combination is thus the adaptation of an old idea or invention (Bevan) using newer technology that is commonly available and understood in the art (the SSR)."

The court found that the claimed invention was but a variation on already known children's toys. This variation presented no nonobvious advance over other toys. The court made clear that there was no technological advance beyond the skill shown in the SSR device. The court found that "[a]ccommodating a prior art mechanical device that accomplishes that goal to modern electronics would have been reasonably obvious to one of ordinary skill in designing children's learning devices. Applying modern electronics to older mechanical devices has been commonplace in recent years."

Example 3:

The claimed invention in *KSR International Co. v. Teleflex Inc.*, 550 U.S. ___, 82 USPQ2d 1385 (2007) was an adjustable pedal assembly with a fixed pivot point and an electronic pedal-position sensor attached to the assembly support. The fixed pivot point meant that the pivot was not changed as the pedal was adjusted. The placement of the sensor on the assembly support kept the sensor fixed while the pedal was adjusted.

Conventional gas pedals operated by a mechanical link which adjusted the throttle based on the travel of the pedal from a set position. The throttle controlled the combustion process and the available power generated by the engine. Newer cars used computer controlled throttles in which a sensor detected the motion of the pedal and sent signals to the engine to adjust the throttle accordingly. At the time of the invention, the marketplace provided a strong incentive to convert mechanical pedals to electronic pedals, and the prior art taught a number of methods for doing so. The prior art (Asano) taught an adjustable pedal with a fixed pivot point with mechanical throttle control. The prior art ('936 patent to Byler) taught an electronic pedal sensor which was placed on a pivot point in the pedal assembly and that it was preferable to detect the pedal's position in the pedal mechanism rather than in the engine. The prior art (Smith) taught that to prevent the wires connecting the sensor to the computer from chafing and wearing out, the sensor should be put on a fixed part of the pedal assembly rather than in or on the pedal's footpad. The prior art (Rixon) taught an adjustable pedal assembly (sensor in the footpad) with an electronic sensor for throttle control. There was no prior art electronic throttle control that was combined with a pedal assembly which kept the pivot point fixed when adjusting the pedal.

The Court stated that "[t]he proper question to have asked was whether a pedal designer of ordinary skill, facing the wide range of needs created by developments in the field of endeavor, would have seen a benefit to upgrading Asano with a sensor." *Id.* at ___, 82 USPQ2d at 1399. The Court found that technological developments in the automotive design would have prompted a designer to upgrade Asano with an electronic sensor. The next question was where to attach the sensor. Based on the prior art, a designer would have known to place the sensor on a nonmoving part of the pedal structure and the most obvious nonmoving point on the structure from which a sensor can easily detect the pedal's position was a pivot point. The Court concluded that it would have been obvious to upgrade Asano's fixed pivot point adjustable pedal by replacing the mechanical assembly for throttle control with an electronic throttle control and to mount the electronic sensor on the pedal support structure.

Example 4:

The claimed invention in *Ex parte Catan*, 83 USPQ2d 1568 (bd. Pat. App. & Int. 2007), was a consumer electronics device using bioauthentication to authorize sub-users of an authorized credit account to place orders over a communication network up to a pre-set maximum sub-credit limit.

The prior art (Nakano) disclosed a consumer electronics device like the claimed invention, except that security was provided by a password authentication device rather than a bioauthentication device. The prior art (Harada) disclosed that the use of a bioauthentication device (fingerprint sensor) on a consumer electronics device (remote control) to provide bioauthentication information (fingerprint) was known in the prior art at the time of the invention. The prior art (Dethloff) also disclosed that it was known in the art at the time of the invention to substitute bioauthentication for PIN authentication to

enable a user to access credit via a consumer electronics device.

The Board found that the prior art "shows that one of ordinary skill in the consumer electronic device art at the time of the invention would have been familiar with using bioauthentication information interchangeably with or in lieu of PINs to authenticate users." The Board concluded that one of ordinary skill in the art of consumer electronic devices would have found it obvious to update the prior art password device with the modern bioauthentication component and thereby gain, predictably, the commonly understood benefits of such adaptation, that is, a secure and reliable authentication procedure.

(G) Some Teaching, Suggestion, or Motivation in the Prior Art That Would Have Led One of Ordinary Skill To Modify the Prior Art Reference or To Combine Prior Art Reference Teachings To Arrive at the Claimed Invention

To reject a claim based on this rationale, Office personnel must resolve the *Graham* factual inquiries. Then, Office personnel must articulate the following:

- (1) a finding that there was some teaching, suggestion, or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings;
- (2) a finding that there was reasonable expectation of success; and
- (3) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

The rationale to support a conclusion that the claim would have been obvious is that "a person of ordinary skill in the art would have been motivated to combine the prior art to achieve the claimed invention and that there would have been a reasonable expectation of success." *DyStar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick Co.*, 464 F.3d 1356, 1360, 80 USPQ2d 1641, 1645 (Fed. Cir. 2006). If any of these findings cannot be made, then this rationale cannot be used to support a conclusion that the claim would have been obvious to one of ordinary skill in the art.

The Courts have made clear that the teaching, suggestion, or motivation test is flexible and an explicit suggestion to combine the prior art is not necessary. The motivation to combine may be implicit and may be found in the knowledge of one of ordinary skill in the art, or, in some cases, from the nature of the problem to be solved. *Id.* at 1366, 80 USPQ2d at 1649. "[A]n implicit motivation to combine exists not only when a suggestion may be gleaned from the prior art as a whole, but when the 'improvement' is technology-independent and the combination of references results in a product or process that is more desirable, for example because it is stronger, cheaper, cleaner, faster, lighter, smaller, more durable, or more efficient. Because the desire to enhance commercial opportunities by improving a product or process is universal-and even commonsensical-we have held that there exists in these situations a motivation to combine prior art references even absent any hint of suggestion in the references themselves. In such situations, the proper question is whether the ordinary artisan possesses knowledge and skills rendering him capable of combining the prior art references." *Id.* at 1368, 80 USPQ2d at 1651.<

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Piezo Film Sensors

Technical Manual

Images SI Inc.

Sensor Products Division

109 Woods of Arden Road

Staten Island NY 10312

Tel: 718.966.3694

FAX: 718.966.3695

Internet: www.imagesco.com

e-mail: imagesco@bellatlantic.net

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INTRODUCTION

Transducer materials convert one form of energy into another, and are widely used in sensing applications. The tremendous growth in the use of microprocessors has propelled the demand for sensors in diverse applications. Today, **PIEZOELECTRIC POLYMER SENSORS** are among the fastest growing of the technologies within the \$18 billion worldwide sensor market. Like any new technology, there have been an extraordinary number of applications where "**PIEZO FILM**" has been considered for the sensor solution. In the 20 years since the discovery of piezoelectric polymer, the technology has matured, practical applications have emerged from a long list of possibilities, and the rate of commercialization of the technology is accelerating.

These documents provide an overview of piezoelectric polymer technology and nomenclature, its properties, and sensor design considerations. It also explores a range of sensor applications that have been successfully developed in recent years.

Solving unique sensor problems is a particular strength of our group of applications engineers. We welcome the opportunity to provide assistance to you during your evaluation of piezo film sensors for your design.

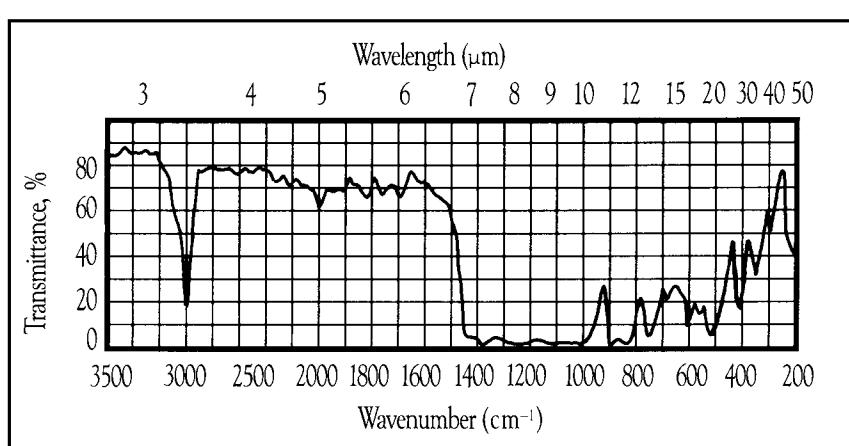
BACKGROUND

Piezoelectricity, Greek for "pressure" electricity, was discovered by the Curie brothers more than 100 years ago. They found that quartz changed its dimensions when subjected to an electrical field, and conversely, generated electrical charge when mechanically deformed. One of the first practical applications of the technology was made in the 1920's by another Frenchman, Langevin, who developed a quartz transmitter and receiver for underwater sound - the first SONAR. Before World War II, researchers discovered that certain ceramic materials could be made piezoelectric when subjected to a high polarizing voltage, a process analogous to magnetizing a ferrous material.

By the 1960's, researchers had discovered a weak piezoelectric effect in whale bone and tendon. This began an intense search for other organic materials that might exhibit piezoelectricity. In 1969, Kawai found very high piezo-activity in the polarized fluoropolymer, polyvinylidene fluoride (PVDF). While other materials, like nylon and PVC exhibit the effect, none are as highly piezoelectric as PVDF and its copolymers.

Like some other ferroelectric materials, PVDF is also pyroelectric, producing electrical charge in response to a change in temperature. PVDF strongly absorbs infrared energy in the 7-20 μ m wavelengths (see Figure 1), covering the same wavelength spectrum as heat from the human body. Accordingly, PVDF makes a useful human motion sensor as well as pyroelectric sensor for more sophisticated applications like vidicon cameras for night

Figure 1. Typical infrared absorption spectrum of PVDF film.



vision and laser beam profiling sensors. A dense infrared array has been recently introduced that identifies one's fingerprint pattern using the pyro effect of piezo polymer.

New copolymers of PVDF, developed over the last few years, have expanded the applications of piezoelectric polymer sensors. These copolymers permit use at higher temperatures (135°C) and offer desirable new sensor shapes, like cylinders and hemispheres. Thickness extremes are possible with copolymer that cannot be readily attained with PVDF. These include ultrathin (200 Å) spin-cast coatings that enable new sensor-on-silicon applications, and cylinders with wall thicknesses in excess of 1200 μ m for sonar. Piezo cable is also produced using copolymer.

PIEZOELECTRIC FILM PROPERTIES

Piezo film is a flexible, lightweight, tough engineering plastic available in a wide variety of thicknesses and large areas. Its properties as a transducer include:

- Wide frequency range—0.001 Hz to 10⁹ Hz.
- Vast dynamic range (10⁻⁸ to 10⁶ psi or μ torr to Mbar).
- Low acoustic impedance—close match to water, human tissue and adhesive systems.
- High elastic compliance
- High voltage output—10 times higher than piezo ceramics for the same force input.
- High dielectric strength—withstanding strong fields (75V/ μ m) where most piezo ceramics depolarize.
- High mechanical strength and impact resistance (10⁹—10¹⁰ Pascal modulus).
- High stability—resisting moisture (<0.02% moisture absorption), most chemicals, oxidants, and intense ultraviolet and nuclear radiation.
- Can be fabricated into unusual designs.
- Can be glued with commercial adhesives.

One major advantage of piezo film over piezo ceramic is its low acoustic impedance which is closer to that of water, human tissue and other organic materials. For example, the acoustic impedance ($Z_o = \rho v$) of piezo film is only 2.6 times that of water, whereas piezo ceramics are typically 11 times greater. A close impedance match permits more efficient transduction of acoustic signals in water and tissue.

Piezo film does have some limitations for certain applications. It makes a relatively weak electromechanical transmitter when compared to ceramics, particularly at resonance and in low frequency applications. The copolymer film has maximum operating/storage temperatures as high as 135°C, while PVDF is not recommended for use or storage above 100 °C. Also, if the electrodes on the film are exposed, the sensor can be sensitive to electromagnetic radiation. Good shielding techniques are available for high EMI/RFI environments.

Table 1 lists typical properties of piezo film. Table 2 provides a comparison of the piezoelectric properties of PVDF polymer and two popular piezoelectric ceramic materials.

Piezo film has low density and excellent sensitivity, and is mechanically tough. The compliance of piezo film is 10 times greater than the compliance of ceramics. When extruded into thin film, piezoelectric polymers can be directly attached to a structure without disturbing its mechanical motion. Piezo film is well suited to strain sensing applications requiring very wide bandwidth and high sensitivity. As an actuator, the polymer's low acoustic impedance permits the efficient transfer of a broadband of energy into air and other gases.

Table 1. Typical properties of piezo film

Symbol	Parameter	PVDF	Copolymer	Units
t	Thickness	9, 28, 52, 110	<1 to 1200	µm (micron, 10 ⁻⁶)
d ₃₁	Piezo Strain Constant	23	11	func {{m/m} over {V/m}}~or~{func {C/m SUP 2} over {N/m SUP}}
d ₃₃		-33	-38	
g ₃₁	Piezo Stress constant	216	162	{func{V/m} over {N/m SUP 2}}~func~\or~{func{m/m} over}
g ₃₃		-330	-542	
k ₃₁	Electromechanical Coupling Factor	12%	20%	
k _t		14%	25-29%	
C	Capacitance	380 for 28µm	68 for 100µm	pF/cm ² @ 1KHz
Y	Young's Modulus	2-4	3-5	10 ⁹ N/m ²
V ₀	Speed of Sound	1.5	2.3	10 ³ m/s
		2.2	2.4	
p	Pyroelectric Coefficient	30	40	10 ⁻⁶ C/m ² °K
ε	Permittivity	106-113	65-75	10 ⁻¹² F/m
ε/ε ₀	Relative Permittivity	12-13	7-8	
func {	Mass Density	1.78	1.82	10 ³ kg/m ³
rho	Volume Resistivity	>10 ¹³	>10 ¹⁴	Ohm meters
R _{SUB}	Surface Metallization Resistivity	<3.0	<3.0	Ohms/square for NiAl
		0.1	0.1	Ohms/square for Ag Ink
tan δ _e	Loss Tangent	0.02	0.015	@ 1KHz
	Yield Strength	45-55	20-30	10 ⁶ N/m ² (stretch axis)
	Temperature Range	-40 to 80...100	-40 to 115...145	func{DEG C}
	Water Absorption	<0.02	<0.02	% H ₂ O
	Maximum Operating Voltage	750 (30)	750 (30)	V/mil(V/µm), DC, @ 25 °C
	Breakdown Voltage	2000 (80)	2000 (80)	V/mil(V/µm), DC, @ 25 °C

Table 2. Comparison of piezoelectric materials

Property	Units	PVDF Film	PZT	BaTiO ₃
Density	10 ³ kg/m ³	1.78	7.5	5.7
Relative Permittivity	ϵ/ϵ_0	12	1,200	1,700
d_{31} Constant	(10 ⁻¹²)C/N	23	110	78
g_{31} Constant	(10 ⁻³)Vm/N	216	10	5
k_{31} Constant	% at 1 KHz	12	30	21
Acoustic Impedance	(10 ⁶)kg/m ² -sec.	2.7	30	30

OPERATING PROPERTIES FOR A TYPICAL PIEZO FILM ELEMENT

The DT1 element is a standard piezo film configuration consisting of a 12x30 mm active area printed with silver ink electrodes on both surfaces of a 15x40 mm die-cut piezo polymer substrate.

1. Electro-Mechanical Conversion

(1 direction) 23×10^{-12} m/V, 700×10^{-6} N/V
 (3 direction) -33×10^{-12} m/V

2. Mechano-Electrical Conversion

(1 direction) 12×10^{-3} V per microstrain, 400×10^{-3} V/ μ m, 14.4V/N
 (3 direction) 13×10^{-3} V/N

3. Pyro-Electrical Conversion

8V/°K (@ 25 °C)

4. Capacitance

1.36×10^{-9} F; Dissipation Factor of 0.018 @ 10 KHz; Impedance of 12 KΩ @ 10 KHz

5. Maximum Operating Voltage

DC: 280 V (yields 7 μ m displacement in 1 direction)
 AC: 840 V (yields 21 μ m displacement in 1 direction)

6. Maximum Applied Force (at break, 1 direction)

6-9 kgF (yields voltage output of 830 to 1275 V)

Electrical to Mechanical Conversion

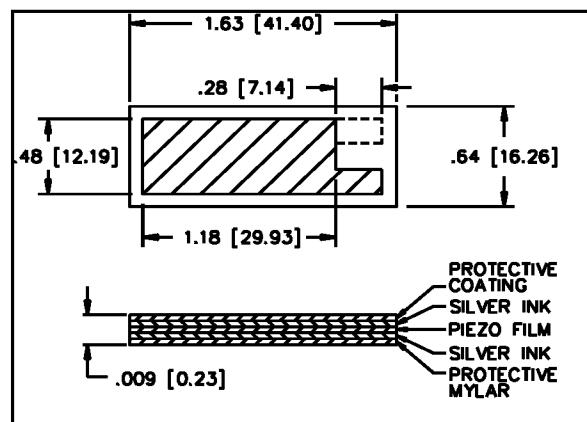
Large displacements of forces are not generally available from piezo film. This becomes apparent when designing loudspeaker elements for instance, as low frequency performance (below 500Hz) tends to be limited. Even a large sheet of film is unable to create high amplitude pressure pulses as low audio frequencies. This does not apply, however, to low to high frequency ultrasonic frequencies, as seen in current designs for ultrasound air ranging transducers (40-50 KHz) and in medical ultrasonic imaging applications. In enclosed air cavities (headset speakers, hearing aids), the low frequency response of piezo film is excellent. For air ranging ultrasound, the piezo film element height controls vertical beam angle and the curvature and width of the transducer controls

horizontal beam pattern. Piezo film air ranging transducers can provide up to 360° field of view, ranging object from a few centimeters to several meters with high resolution.

1. DT1 Element in [mm]

Bimorph configurations (like a bimetal strip) allow the small differential displacement of two reverse-connected elements to be translated into substantial flexural motion. Small fans or optical deflectors can thus be created. Such devices consume very little real power (being capacitive in nature). Large devices may be difficult to drive due to high capacitance, especially when transformers are used to step up the drive voltage. Good amplifier design is important. Nevertheless, conventional fan and blower technologies generate higher flow rates and back pressures than piezo bimorphs.

Although the forces involved are small, the film can be used to excite other mechanical structures over a very wide frequency range. If a second element of film is used to receive the induced vibration, the system can possess a very high dynamic range, even though the overall "insertion loss" due to the film is about -66 dB typically for a structure at resonance. If sufficient gain is applied between these elements, the structure will self-oscillate at its natural frequency. For these resonant mechanical systems, high voltage drive is not required. The amplifier circuit may function adequately from a normal dual rail op-amp supply, or even from a single 9 volt battery. For analysis purposes, even lower applied voltages, e.g., the noise source of a spectrum analyzer at 70 mVrms, are sufficient to insert the mechanical energy into a structure when piezo film is also used to monitor the result.



Mechanical to Electrical Conversion

The sensitivity of piezo film as a receiver of mechanical work input is awesome. In its simplest mode the film behaves like a dynamic strain gage except that it requires no external power source and generates signals greater than those from conventional foil strain gages *after* amplification. Frequency response is thus free from any limitations imposed by the need for high gains and will extend up to the wavelength limit of the given transducer.

The extreme sensitivity is largely due to the format of the piezo film material. The low thickness of the film makes, in turn, a very small cross-sectional area and thus relatively small longitudinal forces create very large stresses within the material. It is easy to exploit this aspect to enhance the sensitivity parallel to the machine axis. If a laminated element of film (for example an LDT1-028K) is placed between two layers of compliant material then any compressive forces are converted into much larger longitudinal extensive forces. In fact, this effect tends to predominate in most circumstances since most substances are compliant to some extent and the ratio of effective sensitivity in the 1 (length) vs 3 (thickness) directions is typically 1000:1.

Piezo film transducers may often cover a much larger area than normal strain gages so any direct comparisons should be performed in a *uniform* strain field for meaningful results. Obviously "point"-type transducers could be used where required although the capacitance of a very small area will require consideration. The low frequency limit of operation will be defined by the greatest resistive load achievable, or by the largest capacitance load that still allows the signal to be easily detected. Operation down to fractions of Hz can be achieved using either conventional charge amplifiers or, since signal levels are relatively high, simple high impedance FET buffer circuits.

Pyro to Electrical Conversion

Piezo film absorbs strongly in the region of 7 to 20 μm which corresponds to well beyond both operating temperature limits of the film. It thus makes a sensitive pyroelectric detectors for, say, human body radiation. Since the pyro sensitivity is strong, care must be taken when designing low (<0.01 to 1Hz) frequency mechanical sensors to avoid ambient temperature changes swamping the output with pyro-generated signal. If a very long time constant is in use, then the film will generate a voltage corresponding to the change in temperature since switch-on. Since the output will be several volts per degree C, substantial offsets may be noticed.

In general, however, most piezo applications will have a cut-off frequency of several Hertz or more. Connecting a device of 1nF capacitance to an oscilloscope input, even at $10\text{ M}\Omega$ impedance, will produce a roll off below 16 Hz. Only a more rapid change in the film temperature will generate a detectable signal.

Common-mode rejection can be used to isolate either very low frequency mechanical strain from simultaneous pyro-effects or vice-versa. These straight-forward techniques are quite familiar to MSI applications engineers who are available for design assistance.

Electrical Design Considerations

A useful model for piezo film which applies for most cases except ultrasonic applications is a strain-dependent voltage source in series with a capacitance. Thus any resistive load will form a divider network with a simple RC high-pass filter characteristic. The cut-off frequency is given by

$$f_{\text{c}} = \frac{1}{2\pi C}$$

and the time constant $\tau = RC$. Operation below the cut-off frequency will give an output signal proportional to the rate of change of the input parameter (differentiator). Application of a constant stress will generate an initial level followed by an exponential decay of rate $\exp(RC)^{-1}$.

A capacitive load will extend the time constant but reduce the magnitude of the response. Energy is always lost when transferring charge from one capacitor to another. Large capacitive loads are useful for attenuating the very large signals arising from powerful impacts—often hundreds of volts.

When driving the film at high voltage and high frequency, the dissipation factor of the film may result in substantial energy loss in the form of heat. Also, the surface resistivity of the electrodes may become significant, especially with vacuum metallized film. Very high localized currents may be encountered. Operation within the field limits given in the Technical Manual is strongly recommended since any arcing will normally destroy the device.

Silver ink, screenprinted onto both film surfaces, has been developed to withstand high voltage and high localized currents. The silver ink metallization has been successfully used in tweeters and active vibration damping applications. The DT1 sample is electroded with the silver ink. The unmetallized border mitigates potential for arcing across the film's thickness. The offset lead attach tabs also preclude high voltage breakdown, as the conductor at each lead attach site is on one side only.

Mechanical Design Considerations

The output energy is proportional to the volume of film stressed. Film thickness may be chosen to optimize the electrical signal or in view of mechanical strength considerations. Thicker films generate higher voltages but form smaller capacitors, so a laminate of thinner film with a compatible, passive material such as polyester (i.e. the LDT1-028K) may be preferable to a single thicker film. Any area

of film that is not undergoing stress will act as a capacitive load on the "active" area and should be minimized if required.

Most metallizations are subject to corrosion, especially when handled. Thin conformal coatings or laminates are frequently applied to maintain surface quality. Acrylics adhesives, synthetic rubber resins, epoxies and cyano-acrylates are all frequently employed in lamination and assembly.

Some designs may use external metallic or conductive substrates as the electrodes, in which case unmetallized film may be used to good advantage. The external metal surface can be in direct contact with the unmetallized film to collect the charge, or, capacitive coupling through thin adhesive tapes or epoxy layers can be employed for ac applications. Patterning of the electrodes is especially useful for defining specific active areas on a continuous sheet and also to allow die-cutting of elements with a clear border around the cut area. Displacement (offset) of upper and lower electrode tabs at the connection point is good practice to prevent unpredictable piezo behavior in this area caused by the influence of the wire terminations. This also allows low cost penetrative lead-attach methods to be used (crimps or eyelets).

Joint Electrical and Mechanical Design Considerations

The capacitive nature of piezo film devices implies that they are susceptible to Electro Magnetic Interference (EMI). This becomes increasingly more important as the output signal level drops. EMI can be ignored where the output is high or when the film is being driven in a non-critical environment. A.C. mains interference may become a problem with unshielded devices. Another potential problem exists when one electrode element is being driven and an another is receiving the vibration signal. Care must be taken to avoid "crosstalk".

Use of ready-made shielded elements (SDT1-028K) supplied with coaxial cable eliminates these problems, but simple measures may be taken with any device to avoid interference.

Unwanted frequencies may be filtered out electronically. If the sensor is to be mounted on a conductive substrate, then this may form one half of a grounded envelope, with the outer electrode forming the other half. Lightweight shielded cable is readily available and is an alternative to twisted pair wires. Attention should be paid to the point of connection itself as this is also an area of EMI vulnerability.

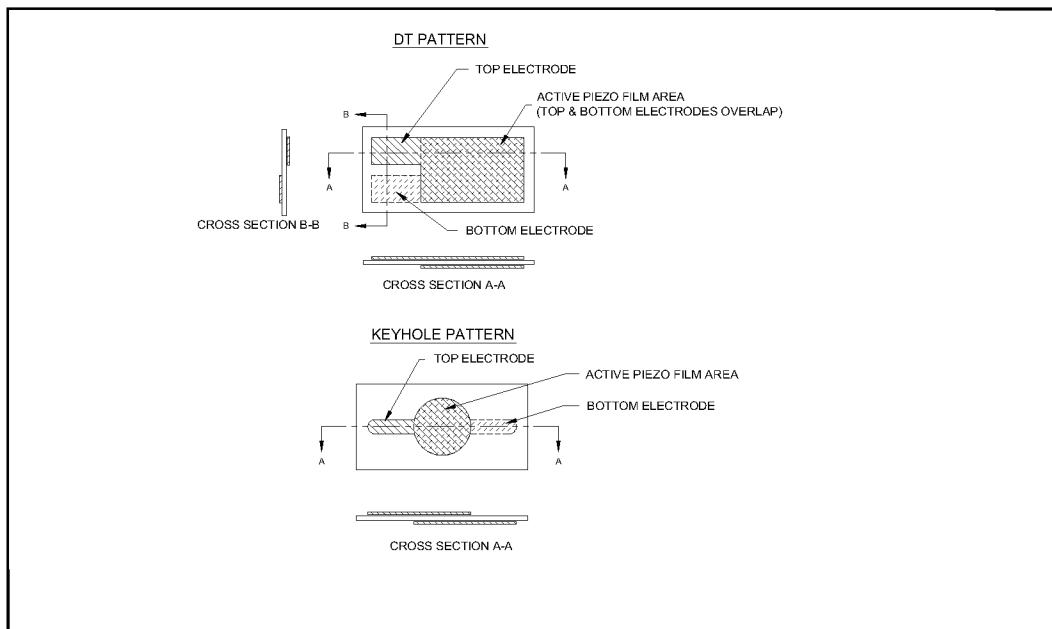
Durable lead attachment techniques have been fully developed by MSI, and most products are supplied with leads preattached. As indicated, some form of coaxial cable is often employed and must be interfaced to a very thin flexible material. Reinforcement at the lead attach site may be required, which can introduce some acoustic effects into the transducer if the interconnection site is free to vibrate.

Thin copper foil backed with a conductive adhesive can provide excellent but non-permanent connections to the film. An area of 1 cm^2 will give a contact resistance of a few $\text{m}\Omega$ s. Crimp-through connectors as used for flexible circuits are routinely used with offset electrode patterns, but thin films require some physical reinforcement for good results. Polyester reinforcement at the lead attach site is a common method to ruggedize the interconnection. The stiffener may lie between the crimp and the electrode with only minor degradation of contact resistance. Typical values are 150-500 $\text{m}\Omega$. Miniature rivets, eyelets and even nuts and bolts, with washers, all combine great strength with good contact resistance at typically less than 100 $\text{m}\Omega$. These techniques may be used to connect to cables using solder tags, or direct onto printed circuit boards.

Clamping methods, either direct to the conductive traces on the PCB or using conductive rubber, ZEBRA® connectors, lugs and washers have all been used with success. Direct connection using silver-loaded (conductive) epoxy also works well, but requires curing time, often at elevated temperature, for best results.

As indicated earlier, other materials may form the electrodes themselves, such as PCB traces or conductive rubber. Capacitive coupling through thin adhesive layers is practical under some a.c. circumstances, allowing some unusual transducer designs with apparently no lead attachment at all!

ZEBRA is a registered trademark of Fujipoly.



LEAD ATTACHMENT TECHNIQUES FOR PIEZO FILM SENSORS

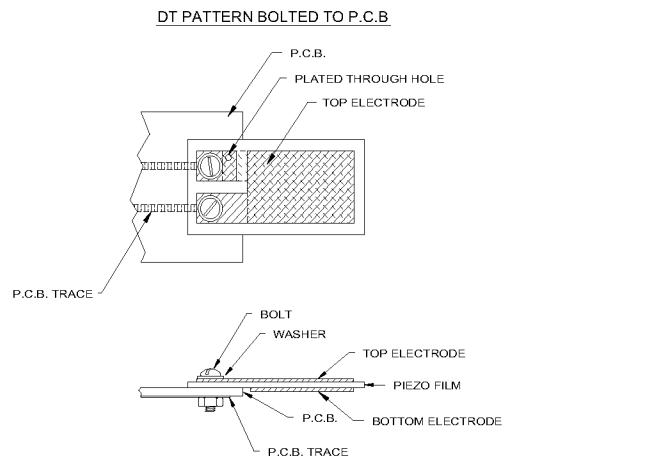
Introduction

Figure 4. Typical piezo film patterns

How to make reliable interconnection to piezo film is one of the most frequently asked customer questions. With this in mind, MSI has paid great attention to the development of techniques to simplify interconnection to piezo film elements. Today, most of the sensor elements supplied to customers from our Division have leads already attached. The aim of this article is to examine and discuss available interconnection options.

Some of the most convenient interconnection techniques require that MSI apply patterned electrodes on one or both surfaces of the

piezo film—this can always be done to customers' requirements during manufacture—alternatively, a simple method achieving the same goal is presented at the end of the text. In general, patterned electrodes are achieved during piezo film manufacturing by screen printing conductive inks, metal masking during sputtered electrode deposition, or chemically etching patterns by photolithographic techniques.



The Targets

Considered here are the design objectives desired for the lead-attach method. Not all objectives can be achieved with any one technique. Designers should identify the most important objectives and select among the interconnection options accordingly.

- High conductivity/low resistance — surprisingly, high conductivity interconnection is not a particularly important parameter for most piezoelectric applications. Piezo transducers are frequently used in high-impedance circuits where inclusion of a few ohms does not usually affect performance. More important, however, is consistence—the resistance should not fluctuate during use since this will introduce a source of electrical noise.
- Low mass — this is especially important when the piezo film is not to be clamped to a mechanical support structure. The acoustic effect created by the mechanical vibration of the mass of the interconnection on an otherwise flexible structure can be dramatic.
- Low profile — many piezo film applications arise by virtue of the low thicknesses of piezo film. Interrupting this with bulk terminations is often prohibited. Contact vibration sensors can show distinct resonances if film is not bonded flush to the contact surface to include the interconnection.
- Flexibility — here again is a property that must often match that of the film itself. Some degree of flexibility is a distinct advantage in many applications.
- Low area — useful piezo devices can be quite literally be employed as "point" receivers. Small piezo-active areas (where the top and bottom conductors fully overlap) can be configured with displaced or off-set lead-attach tabs. The top and bottom tabs are off-set with respect to each other (when viewed through the film thickness). This allows a precisely defined active area

(overlapped electrodes) with non-piezo conductors (off-set tabs) leading to remote bonding sites, a technique most frequently employed for "small" devices.

- Mechanical Strength — very often the greatest strain experienced by a polymer transducer is around the connection, whether by accident (tripping over the cable) or by design. In general, those methods which involve the interconnection penetrating through the film at the off-set tab locations with crimps, eyelets or rivets yield the best ultimate strain resistance. Often the lead attach area is reinforced with polyester to improve the strength of the penetrative interconnection.
- Long-term Stability — including all the usual environmental parameters. Most interconnections have unlimited life (crimps, eyelets, conductive rubber connectors). Others have a more limited shelf life (conductive tapes)
- Speed and Ease of application — of particular importance when high volume production is planned. Many interconnection techniques are supported by semi-automatic equipment for volume production (crimps, eyelets) while others are labor intensive (conductive adhesives).
- Electrical strength — an issue associated mainly with electrically driven (high voltage) elements such as loudspeakers and actuators.

The Design Considerations

Two major issues control the selection of lead-attach methods:

- Is anchorage of the film allowed at the site of lead-attach? This can be a major advantage, for example, direct connection or capacitive coupling to the conductive traces of a printed-circuit board. **Figure 6.**
- Is special patterning of the film available, which would allow penetrative techniques? (with MSI Sensors custom patterning service, the answer is almost always "yes.") Simple experimental methods allow the same result.

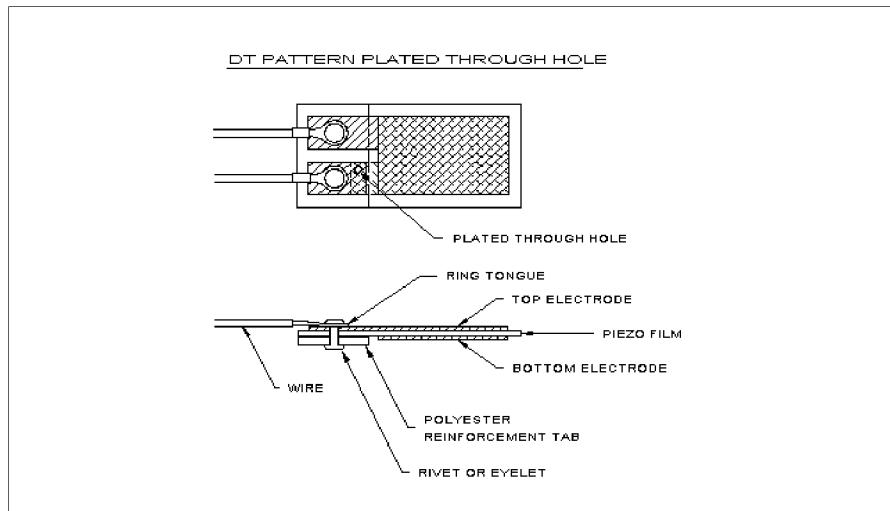
This concludes the "questions" section—now, hopefully, are the "answers."

The Methods

Figure 4.

Penetrative - Here the techniques involve piercing the film (and possibly additional reinforcing laminates to give sufficient thickness and strength), and thus the film should be patterned with a displaced or off-set lead-out arrangement to prevent shorting of upper and lower electrodes by the inserted connector.

- Rivets or eyelets can be affixed to the off-set conductive traces on the piezo film. Included between the eyelet or rivet can be a ring tongue lug terminal with wire attached. The eyelet or rivet mechanically presses the conductive ring against the off-set patterned electrode to make reliable interconnection.
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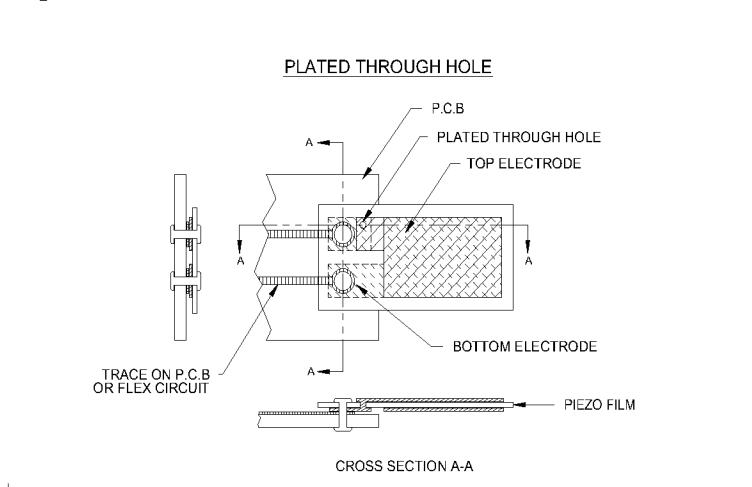


To affix the piezo film directly to a PCB, small "POP" or "blind" rivets or eyelets can be used in conjunction with patterned film electrodes and the conductive tracks on the PCB to allow a single operation to form the interconnection. During screen printing of conductive ink electrodes, a small "plated through hole" can be formed in one of the off-set tabs, thereby bringing both conductors to the same side of the piezo film. This greatly facilitates riveting the film electrode tabs to the corresponding PCB traces. If the "plated through hole" technique is not used, then the top film electrode can be electrically connected by the rivet to a conductive trace on the underside of the PCB. The bottom film electrode is electrically connected to a corresponding trace on the top of the PCB and held in intimate contact by the pressure exerted by the rivet.

- Nuts and bolts - Wires terminated with washers, ring-tongue lugs, solder-tags, etc. can easily be incorporated with small nuts and bolts.
- Crimp Connectors — generally, crimps designed for flexible circuit technology work well with piezo film elements.

Crimps can have solder tabs for affixing wires, or the crimp ends can be inserted into corresponding holes in a PCB and soldered to the underside of the PCB (maximum of a few second soldering time so as not to overheat the film). Like the eyelets mentioned above, crimps are normally designed to work with a specified thickness of "substrate," so film may require "padding" on one side (i.e., polyester reinforcement) to accommodate the crimp connectors. Additionally, a complete multi-way connector may be crimped to a more complex device, giving straight plug-in compatibility with other connectors.

Figure 5.



Non-penetrative (and temporary) - Conductive-adhesive coated Copper Foil Tape (e.g., 3M #1181)—available in widths from 3mm up to 25mm. Best results are obtained by...

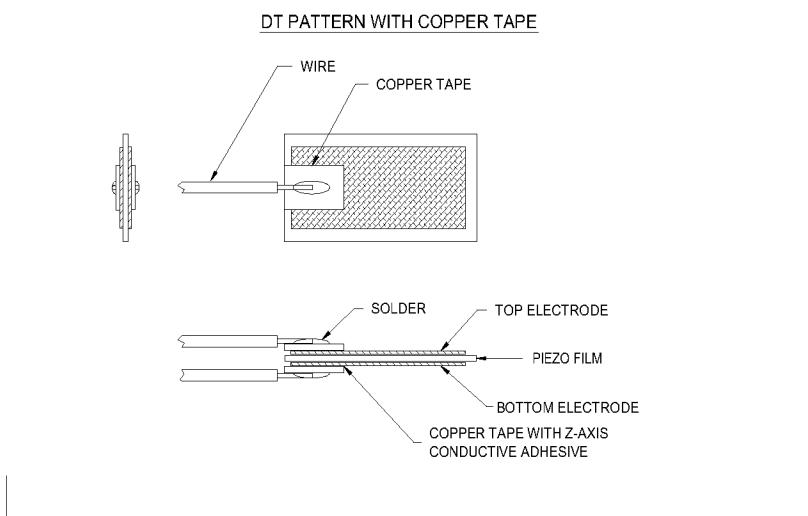
Figure 7.

- Using a "reasonable" area of tape (perhaps about 1cm or more). Small pieces do tend to lift off easily.

- Soldering wires to the tape FIRST, then removing the liner and adhering with gentle pressure to film. If small areas are to be used, solder before cutting the contact pad down to size, thus leaving the excess area to act as a heat sink.

Soldering does appear to degrade the adhesive properties in the vicinity of the joint. NOTE: 3M does not recommend relying on the conductive adhesive in this way and suggest an embossed version of the same tape. The tape is really designed for large area contacts to metal, but results have shown this method to be an effective, if not guaranteed, technique. An aluminum version of this product is available (Part No. 1170). Beware of similar tapes that do not have conductive adhesives (although these can be used for shielding, etc.)

- Conductive Transfer Tape—e.g., 3M #9702 (Preliminary product). An acrylic adhesive layer loaded with conductive particles giving excellent "Z-axis" conductivity (i.e., through the thickness of the tape) with very high resistivity in the X and Y axes. Thus single or multiple-way connections may be made with a single strip. This material is relatively new. Initial results seem very promising. Obviously this can be used to make direct connection with PC board or strip, or to sections of foil with soldered leads.
- Negative aspects are a) high cost, and b) like all transfer adhesives, there is a tendency for the material to adhere to its own liners around the edge so that "stringing" occurs on liner removal. NOTE: Since time of writing, this product has been superseded by an improved version (#9703) with an easy-release liner. This may not yet be generally available.
- Conductive Epoxy. This is usually available in two-part form (adhesive and hardener). Precise metering and mixing of the small quantities usually required is rather difficult and messy. One-part, pre-mixed material is available as a product which is stored at very low temperature and should be used and cured at room temperature. Curing of any epoxy mix can usually be accelerated by use of higher temperature, but since the piezo film has a modest high-temp capability, curing is often a long term process (many hours, a day). Some mechanical clamping is usually required on the parts to be bonded. Final reinforcement with "ordinary" epoxy can be reassuring. Negative aspects: difficulty of use, cure time, higher cost, short "shelf life."
- Low melting-point Alloys—some alloys (e.g., Indium/Tin/Bismuth) which are known as "fusible alloys" rather than "solders," melt at temperatures which allow them to be used on piezo film with suitable metallization (e.g., gold, cooper, silver or silver ink). Rather aggressive fluxes are often required, and the joint may be brittle. Mechanical strength is limited by the adhesion of the metallization onto the film surface, so once again, reinforcement with epoxy may help. For joints that must be very small and do not need undue mechanical strength this may



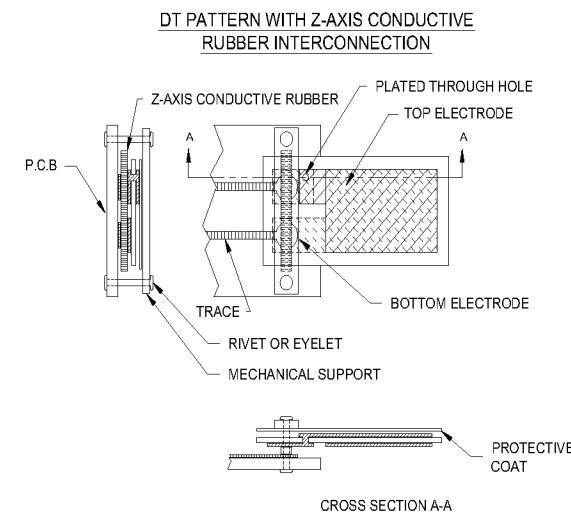
prove a valuable technique. Negative aspects: only certain metallizations are appropriate, sample quantities hard to come by. Mechanical strength limited [Indium Corp.]

- Zebra® Connectors — Conductive rubber spliced with insulating rubber as used to form contacts to LCD displays. High density multiple-way contacts may be made. External clamping of contacts is required.
- Mechanical clamping—simply sandwiching the film between two conductive surfaces (possibly using a thin layer of conductive-loaded rubber) can provide excellent results. Two rings can provide useful support for diaphragms, speakers, etc.

3.

Mechanical clamping—simply sandwiching the film between two conductive surfaces (possibly using a thin layer of conductive-loaded rubber) can provide excellent results. Two rings can provide useful support for diaphragms, speakers, etc.

- Capacitive Coupling - In certain applications, no metal electrode is required on the piezo film itself. Thin, non-conductive adhesives can affix the unmetallized film to a conductive surface. The conductive surface in effect provides the film's electrodes in ac applications. A PCB, having conductive pads on one surface corresponding to the desired active sensor area, is an embodiment of this concept. The opposite piezo film surface can be metallized with a ground electrode. The film can be sandwiched between two conductive surfaces with or without adhesive to form electrodes.



User Etching of Piezo Film Electrodes

Patterned electrodes are available from MSI in either silver screen printed ink or as sputtered electrodes. In some instances, customers purchase fully metallized sheets for experimentation, and want to produce their own patterns. This is very difficult with screen printed inks as they cannot be easily etched or mechanically braided. For sputtered electrodes, standard photolithographic techniques work quite well.

In order to pattern piezo film in such a way as to allow penetration of film without shorting top and bottom electrodes, a very simple technique may be employed which works on any vacuum deposited electrodes (NOTE: not recommended for Ag Ink.)

One terminal of a power source (bench p.s.u. or 9 volt battery) is connected via a conductor pad or block by mechanical pressure to the piece of film in question. The other terminal is brought to a conductive point (needle, wire-end, blunt scalpel, etc.) and the area required to be isolated simply drawn around. Sufficient current normally passes to cause arcing at the point contact and the metallization is vaporized. Concentric "guard rings" may be drawn for extra confidence.

For more complex patterning of thin sputtered metallization, it is possible to coat the piezo film with photoresist aerosol (both sides if necessary). The cured spray can then be exposed through a mask using UV light, as with conventional PCB techniques, and then dipped in an etchant. Complete etching of the very thin metal layer occurs in seconds.

Copper/Nickel metallizations etch very well with standard PCB etchant (ferric chloride). Other metals require special etchants for good results (Aquaregia for gold). Remember that the metallization layer may only be a few hundred atoms thick (300-700 Å), and therefore fine traces are very vulnerable to scratching or cracking.

High Voltage Techniques

The use of piezo film as a vibration exciter requires separate consideration. Since the impedance of a capacitive transducer decreases with frequency and approaches infinity for low frequencies, very high voltages (a few hundred volts typically) may be required to drive, for example, full audio-range loudspeakers. Frequently, transformers are used to step up moderate voltages to supply the required drive signal. Under these circumstances, extreme stresses may be placed upon the connections. Consider first applying a voltage step of 30V to a capacitor of 100nF with an overall circuit resistance of 2 ohms. The initial current pulse peaks at 15 amps (assuming the supply is capable of supplying this). Such a current "spike" may well show up defects in connectors.

Consider next a transformer which steps 12V signals up to 240V. A DC current in the primary of 200 μ A (corresponding to an applied voltage of 0.5 volts), when broken, may cause a voltage surge of 830 volts across the secondary circuit, well in the excess of the expected X 20 magnification factor. Even with heavy capacitive loading, high voltages may be seen. Worse still, if the secondary circuit is broken, current pulses exceeding 60A with durations of only tens of nanoseconds may arise. Such phenomena should not trouble well-formed connections. But if a lead-attach method has been used which has any trapped air, the effect of the reduced dielectric constant may be to promote breakdown. Such events may be catastrophic, as the familiar crackling sound and lively blue sparks will testify.

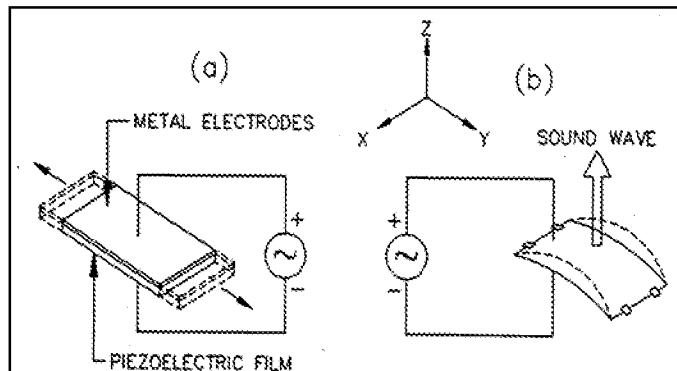
Solutions are:

1. Silver ink electrodes are a must - the thin sputtered electrodes cannot withstand the high voltages
2. Large area contacts to reduce stress. We paint silver ink around eyelets/rivets to provide extra conduction paths to the film electrode.
3. (Possibly) a semi-resistive contact pad to reduce current surges—equivalent to including a series resistance in the circuit. Practical values up to about 1 k will produce only a fractional loss in output and will reduce the magnitude of current spikes.

Figure 10. Clamped film in d_{31} mode produces sound

FREQUENCY RESPONSE

Unlike piezo ceramic transducers, piezo film transducers offer wide dynamic range and are also broadband. These wide band characteristics (near dc to 2GHz) and low Q are partly attributable to the polymers' softness. As audio transmitters, a curved piezo film element, clamped at each end, vibrates in the length (d_{31}) mode, as shown in Figure 10. Piezo film is a very high fidelity tweeter, also used in novelty speakers for toys, inflatables and apparel. The d_{31} configuration (Figure 10) is also used for air ultrasound ranging applications up to frequencies of about 50 KHz.



When used as a high ultrasonic transmitter (generally >500 KHz), piezo film is normally operated in the thickness (d_{33}) mode. Maximum transmission occurs at thickness resonance. The basic half-

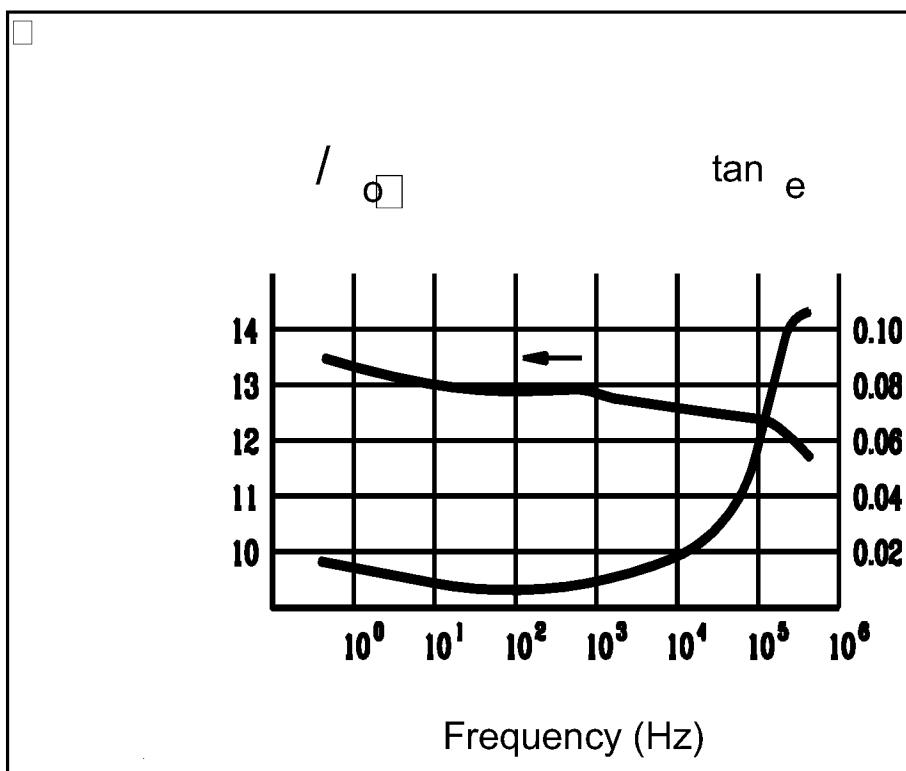
wavelength resonance of 28 μ m piezo film is about 40 MHz:

$$f_r \sim \frac{v}{2t} \sim \frac{2.2 \times 10^3 \text{ m/sec}}{2 \times 28 \times 10^{-6} \text{ m}}$$

Figure 11. Dielectric permittivity and dissipation factor vs. frequency

Resonance values thus depend on film thickness. They range from low MHz for thick films (1,000 μ m) to >100MHz for very thin films.

Figure 11 shows the effect that frequency has on permittivity and dissipation factor at room temperature. As a result of its very low permittivity ϵ (1 percent that of piezo ceramics), the film exhibits g-constants (voltage output coefficients) that are significantly greater than piezo ceramics ($g = d/\epsilon$).



PIEZO FILM AT LOW FREQUENCIES

Introduction

The behavior of a piezo film component at low frequencies is fairly straightforward to describe in electrical terms, yet is quite frequently misunderstood. Since any practical application of the technology will most likely involve some consideration of this topic, it is the intent of this article to examine the subject at some length. The treatment is made as non-mathematical as possible, with verbal descriptions and real-world examples being used to illustrate the concepts. Some familiarity with the use of FFT techniques to transform between time-domain and frequency-domain descriptions is assumed, but not essential.

Connecting Up

In most instances, the first evaluation of piezo film begins with connecting a piezo component to an oscilloscope via a probe ("scope probe"). Under normal electronics circumstances, a scope probe can be considered to be an "infinite impedance" - so high, that its effect on the circuit under test can be neglected. **Not so with piezo film** - in many cases, a scope probe can act almost like a short-circuit. Typical probes, when plugged in to an oscilloscope, have an effective *resistance* of $1M\Omega$ (one million ohms). Others may be fixed at $10M\Omega$, while many are conveniently switchable between "x1" ($1M\Omega$) and "x10" ($10M\Omega$). Note that the physical element comprising the $1M\Omega$ resistance is usually built into the oscilloscope input stage, rather than being a discrete component within the probe itself. A "x1" probe is thus basically a length of shielded cable with suitable contacts attached to each end.

Source Capacitance

To analyze what will happen when the probe is connected, we now need to consider the properties of the piezo film element. Perhaps the most important characteristic (after the piezoelectric property, of course) is the material's *capacitance*. Capacitance is a measure of any component's ability to store electrical charge, and is always present when two conductive plates are brought close together. In our case, the conductive plates are the conductive electrodes printed or metallized onto each surface of the film. The capacitance of the device is strongly affected by the properties of the insulator serving to space the plates apart, and the measure of the insulator's capacity to store charge is given by its *dielectric constant* or *permittivity*.

PVDF has a high dielectric constant compared with most polymers, with its value being about 12 (relative to the permittivity of free space).

Obviously, the capacitance of an element will increase as its plate area increases, so a large sheet of film will have a larger capacitance than a small element. Capacitance also increases as the film thickness *decreases*, so for the same surface geometry, a thin film will have a higher capacitance than a thick film.

These factors are formally related in the equation:

$$\text{func } \{C = ``\text{varepsilon}_r" \text{ ``A OVER t"}\}$$

where C is the capacitance of the film,

ϵ_r is the permittivity (which can also be expressed in the form

$\epsilon = \epsilon_r \epsilon_0$ where ϵ_r is the relative permittivity (about 12 for PVDF), and ϵ_0 is the permittivity of free space (a constant, $8.854 \times 10^{-12} \text{ F/m}$)

and A is the active (overlap) area of the film's electrodes
and t is the film thickness

Table 3. Capacitance values of common piezo film components

The units of capacitance are Farads (F), but usually much smaller sub-multiples are encountered: microfarads (μF or 10^{-6} F), nanofarads (nF or 10^{-9} F) and picofarads (pF or 10^{-12} F).

The capacitance of any piezo film element can be calculated using the formula, or measured directly using a hand-held capacitance meter, or bench-top instrument such as an "LCR bridge".

Capacitance values should be quoted at a given measurement frequency - where this is not given, a frequency of 1 KHz is often assumed. Capacitance values of piezo film components usually decrease as the measurement frequency increases.

Description	Part No.	Capacitance
LDT0-028K/L	0-1002794-1	500 pF
DT1-028K/L	1-1002908-0	1.3 nF
DT1-052K/L	2-1002908-0	650 pF
DT2-028K/L	1-1003744-0	2.6 nF
DT4-028K/L	1-1002150-0	9 nF
8" x 11" 28 μm	1-1003702-4	30 nF
HYD-CYL-100	0-1001911-1	43 pF

Figure 12. Piezo film element as a simple voltage generator

Equivalent Circuit of Piezo Film

We are now ready to draw out an electrical equivalent of the piezo film element. There are two equally valid "models" - one is a *voltage* source in series with a capacitance, the other a *charge* generator in parallel with a capacitance - but the latter is uncommon in electrical circuit analysis and we will concentrate on the voltage source (see Figure 12).

The dashed line represents the "contents" of the piezo film component. The voltage source V_s is the piezoelectric generator itself, and this source is directly proportional to the applied stimulus (pressure, strain, etc). It is not the purpose of this article to elaborate further on the calculations involved, but it is important to realize that this voltage will absolutely follow the applied stimulus - it is a "perfect" source.

Note, however, that the node marked "X" can never be accessed! The film's capacitance C_0 will always be present and connected when we monitor the "output" of the film at the electrodes.

Adding in a resistive load

Now we can add in the effect of connecting up to the oscilloscope. The oscilloscope and its probe are modeled simply as a pure resistance, although in reality there will be a very small capacitance associated with the probe and the cable (usually in the region of 30 to 50 pF). This can be neglected if the film capacitance is significantly higher in value.

The voltage measured across the load resistor R_L will **not** necessarily be the same voltage developed by the "perfect" source (V_s).

To see why, it is helpful to redraw this circuit in another way.

Potential Divider

With the circuit shown in Figure 13 redrawn as in Figure 14, it is easier to see why the full source voltage does not always appear across the resistive load.

A *potential divider* is formed by the *series* connection of the capacitance and the resistance. Since the capacitance has an impedance which varies with frequency, the share of the full source voltage which appears across R_L also varies with frequency.

The proportion (V_L) of V_s which appears across R_L is given by:
where

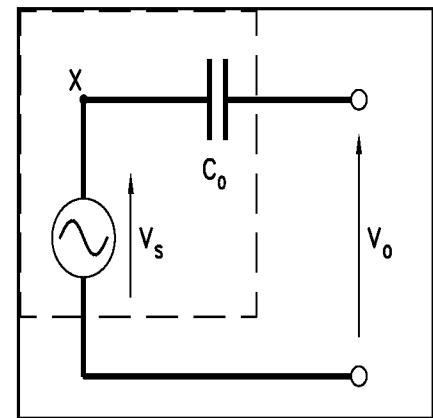
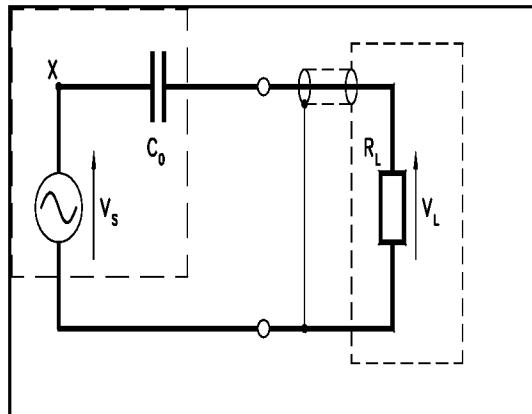
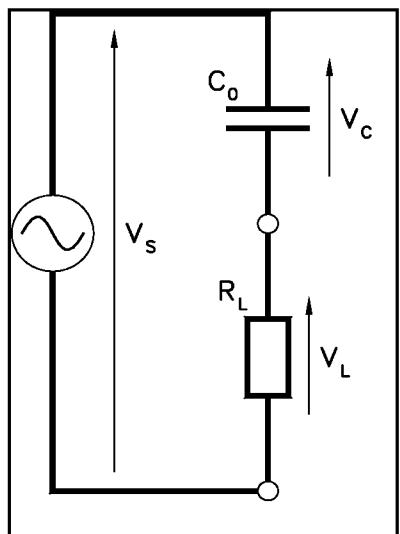


Figure 13. Adding the oscilloscope as resistive load





$$\text{func } \{ Z \text{ SUB } C = -jX \text{ SUB } C = -j \text{ OVER } \{ 2\pi f C \} \}$$

$$\text{func } \{ V \text{ SUB } L = R \text{ SUB } L \text{ OVER } \{ R \text{ SUB } L + Z \text{ SUB } C \} \}$$

(j denoting $\sqrt{-1}$, and X_C being the reactance of the capacitive element. For simplicity, we ignore any resistive component of the film's impedance).

Figure 14. Potential divider

The above equations may be used in simple ways to calculate the voltage level expected to be observed in simple cases where the frequency of excitation is constant, and so a value of f can simply be substituted. In many real-world cases, however, there may be a distribution of signal energy over a band of frequencies. Then it becomes useful to consider the "frequency response" of the network.

Figure 15. Magnitude response of R-C filter

Frequency Response

This is illustrated in the following example graphs. First, a lin/lin plot is shown (Figure 15, linear y-scale or amplitude, plotted against linear x-scale or frequency) with the corresponding phase plot (Figure 16) also shown in lin/lin form. Following these is a log/log plot (Figure 17), which will be dealt with in a little greater detail.

Note that the phase curve indicates that at very low frequencies, the observed voltage will show significant phase deviation from the source (limiting at -90° or $-\pi/2$ radians at "dc" or zero Hz). The significance of this effect is

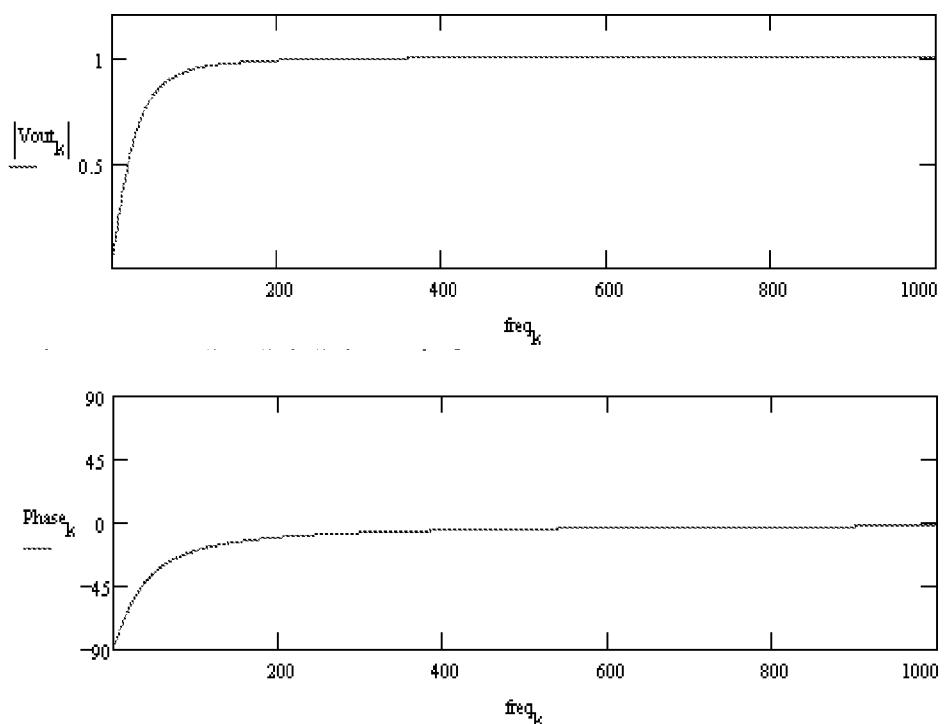
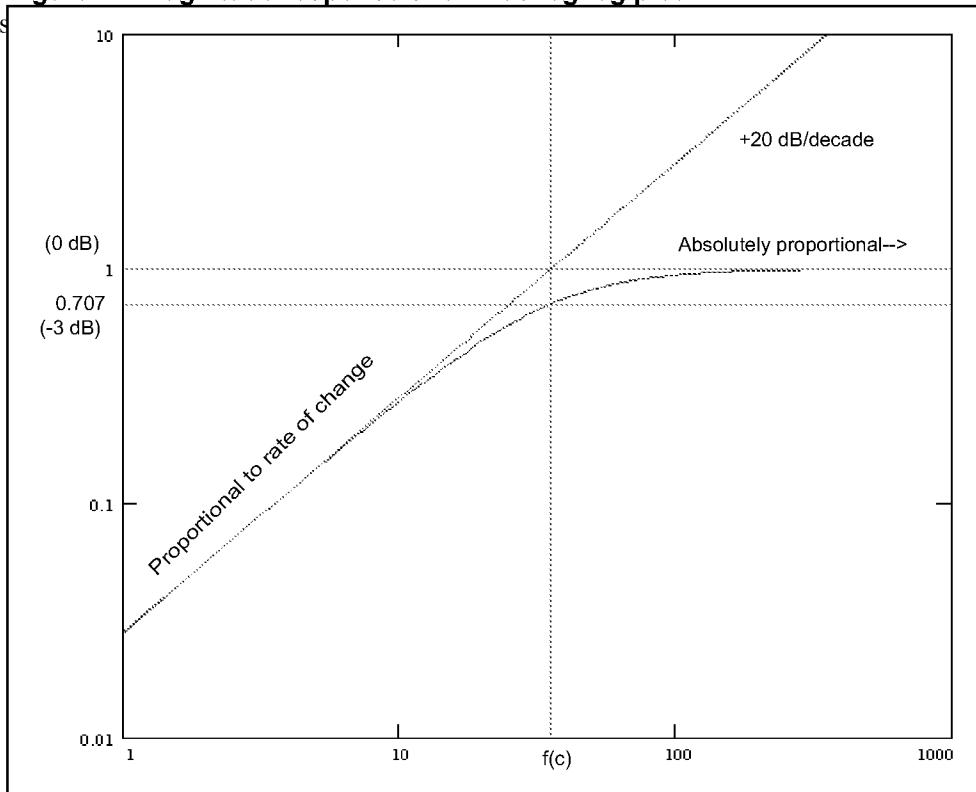


Figure 17. Magnitude response shown as log/log plot



Analysis of the log/log R-C frequency response curve

Some key features:

- the overall characteristic of this network is known as a high-pass filter
- the frequency at which the magnitude falls to 0.707 or -3 dB is known as the "cut-off" or "corner" frequency of the high-pass filter
- this frequency can be calculated as $f(c) = 1/(2\pi RC)$, when both the resistance R and capacitance C are known
- at frequencies well *below* the cut-off frequency, the plot has the form of a straight line with gradient +20 dB/decade (in other words, doubling the frequency will double the signal amplitude) - this characteristic is identical with that of a *differentiator* network, and gives an output which is proportional to the *rate of change* of the input quantity
- at frequencies well *above* the cut-off frequency, the plot is level at "unity gain" and the output is directly proportional to the input quantity
- the filter characteristic can be approximated by these two intersecting straight lines, but the magnitude actually follows an asymptotic curve, with magnitude -3 dB at the cut-off frequency where the straight lines cross
- the filter characteristic can then be applied to the frequency-domain description of any practical signal by multiplying the filter transfer characteristic with the spectrum of the input signal, and deriving a response curve (output) which can in turn be transformed back into a time-domain signal.

Some practical examples of the effect of this filter characteristic will be shown next. For each signal, the time-domain description of the "perfect source" (e.g. the waveform which would be seen on an oscilloscope if the filter characteristic was absent) is given first, followed by its spectrum (obtained by use of the FFT [Fast Fourier Transform] algorithm supplied in the analysis software), then the filter characteristic (identical for all examples, but shown to emphasize the effect), then the resulting output signal spectrum obtained by multiplying the complex input spectrum by the complex filter characteristic, and finally the corresponding time-domain description obtained by inverse FFT, which shows the waveform an engineer would expect to observe in reality.

Note: in Figures 15, 16 and 17 the R-C values used to generate the curve were $R = 1M\Omega$ and $C = 4.5 \text{ nF}$. In the following plots, the value of C was reduced to 1.5 nF. These values were chosen somewhat arbitrarily to demonstrate the principle, and so the scaling on the curves has not been annotated. But the time waveforms can be read in x units of seconds, and the frequency curves with x units of Hz. The cut-off frequency for $R = 1M\Omega$ and $C = 1.5 \text{ nF}$ is approximately 106 Hz.

Key to following figures

Figure 18 shows a relatively high-frequency sine wave passing through the network. In the input spectrum, the signal is represented by a single spectral line at the appropriate frequency. This frequency is just below the filter "cut-off", and so is only slightly attenuated by the network. The resulting output wave is diminished in amplitude, and slightly shifted in phase.

Figure 19 shows the same process applied to a slower sine wave. In this case, the attenuation is much greater, and the phase shift more significant. This situation occurs when trying to monitor steady vibration at "too low" a frequency using a piezo sensor. The phase behavior may be significant if a control loop is to be implemented.

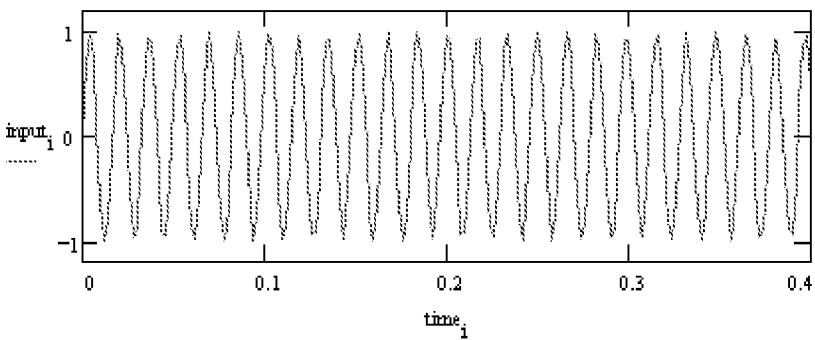
Figure 20 shows a harmonic series, with a number of discrete spectral lines all lying below the cut-off frequency. Each is attenuated to a different extent, and so the "balance" of harmonics in the output signal is altered.

Figure 21 shows a slow half-sine input pulse (typical of many mechanical impact signals). Although the high-frequency content is largely unaltered, the output waveform appears heavily "distorted" and clearly shows both positive and negative excursions, whereas the input waveform is unipolar.

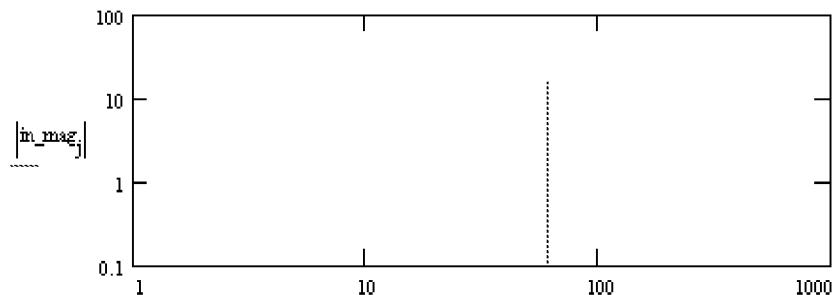
Figure 22 shows a sawtooth waveform with slowly rising "leading edge" followed by a "snap" descent back to zero. Many piezo switches detect this form of mechanical event. In the output waveform, the "leading edge" has almost disappeared, but the "snap" gives almost full amplitude. Note the polarity of the output pulse relative to the input waveform.

Figure 18. Effect of R-C filter on High Frequency Sine Wave input waveform

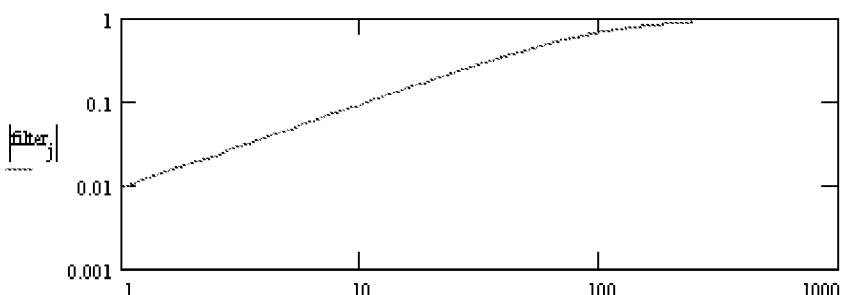
a) Input waveform



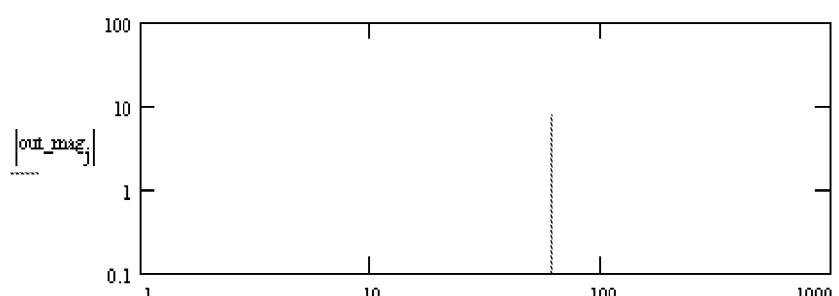
b) Input spectrum



c) Filter characteristic



d) Output spectrum



e) Output waveform

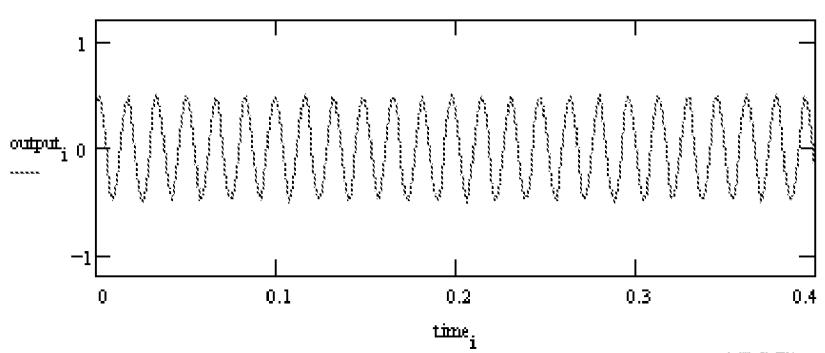
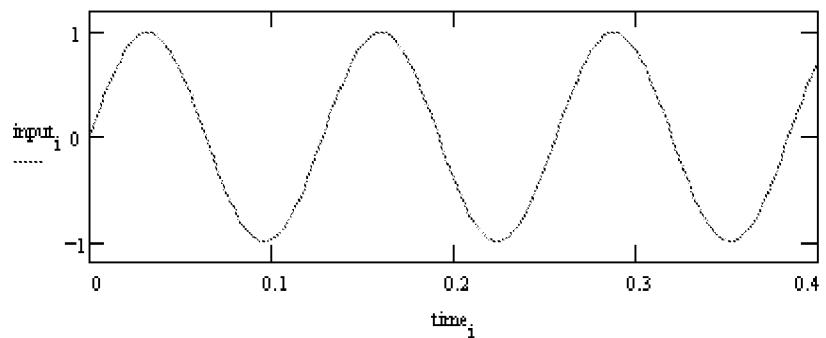
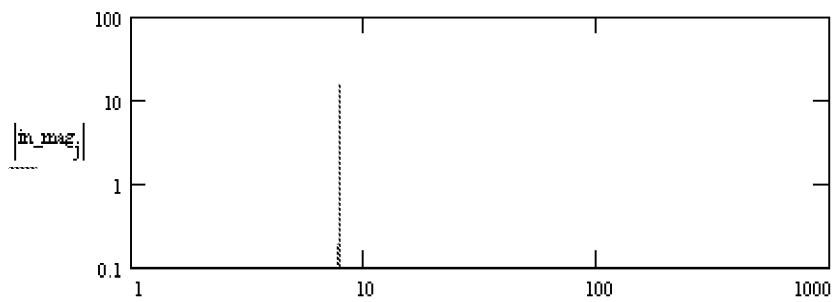


Figure 19. Effect of R-C filter on Low Frequency Sine Wave input waveform

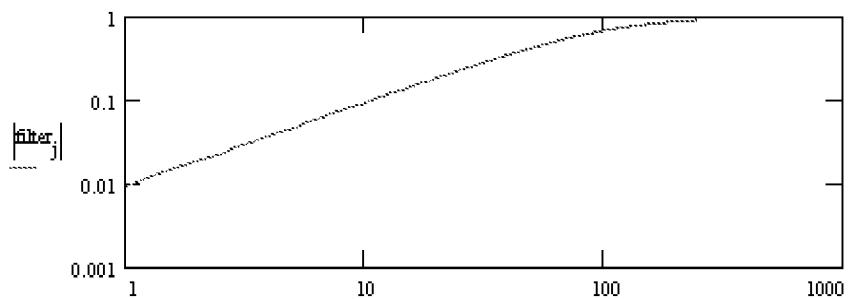
a) Input waveform



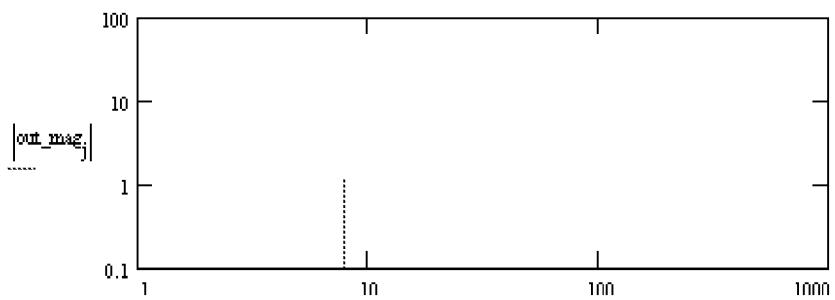
b) Input spectrum



c) Filter characteristic



d) Output spectrum



e) Output waveform

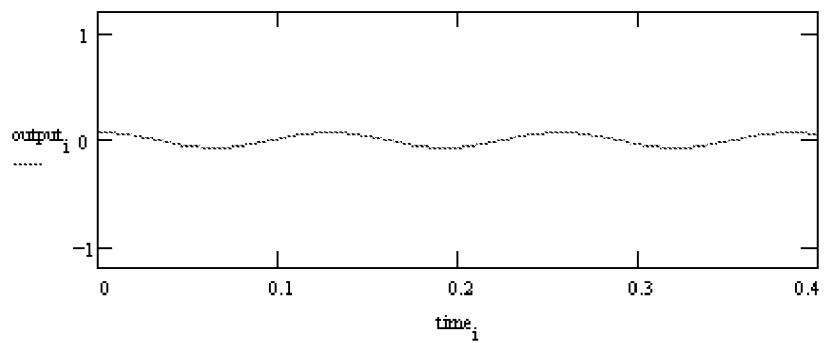
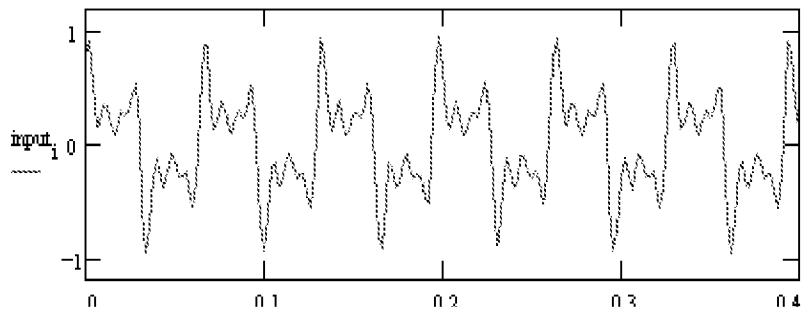
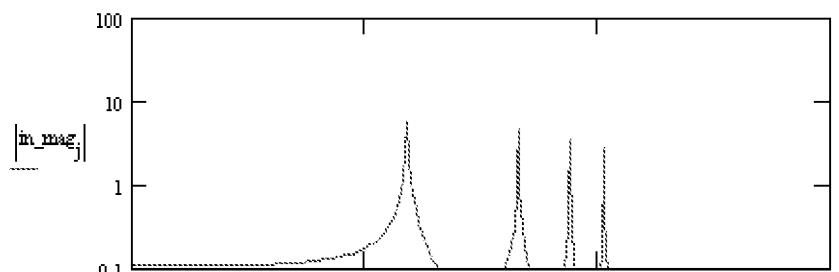


Figure 20. Effect of R-C filter on Harmonic Series input waveform

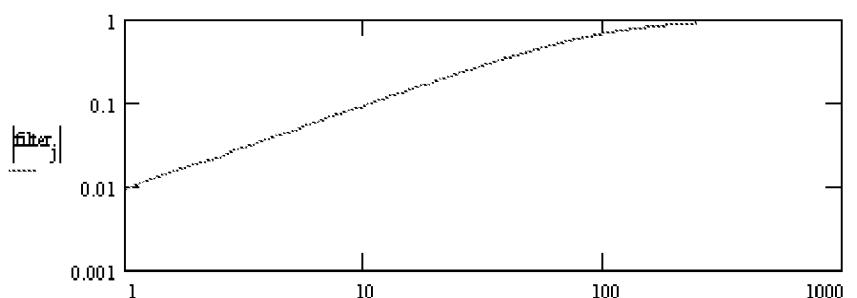
a) Input waveform



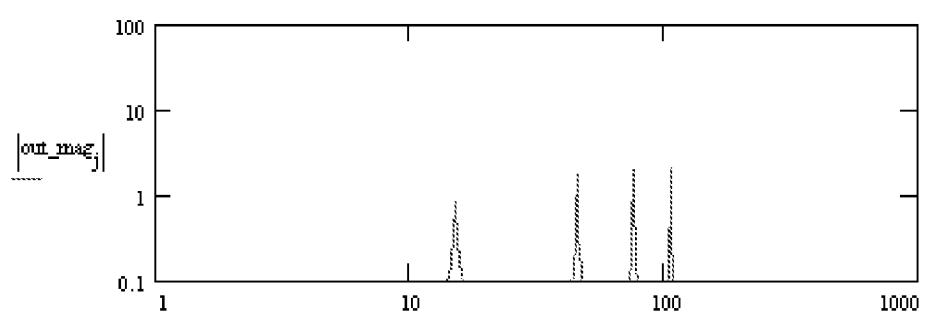
b) Input spectrum



c) Filter characteristic



d) Output spectrum



e) Output waveform

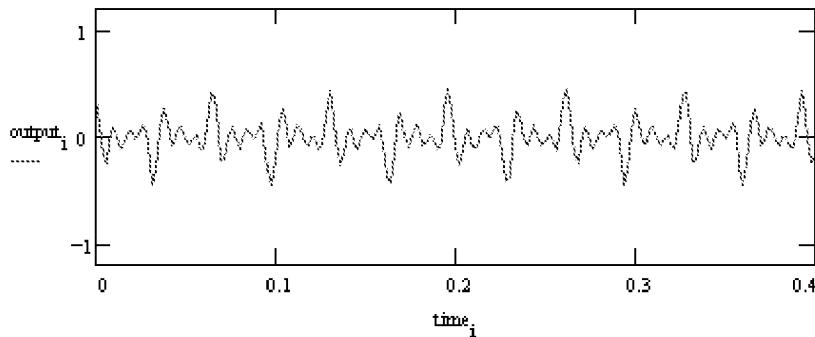
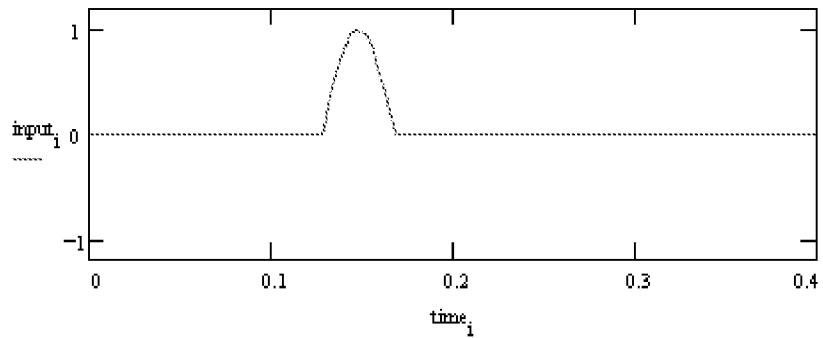
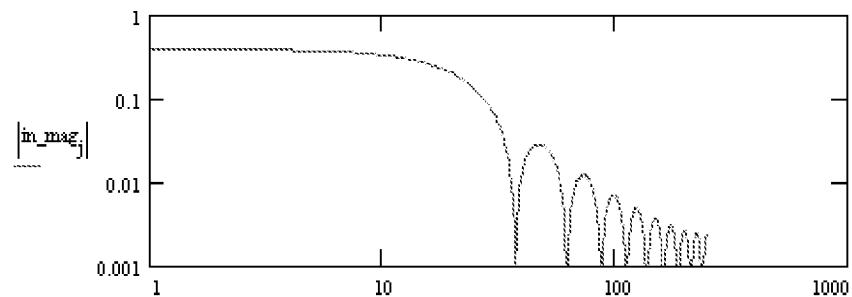


Figure 21. Effect of R-C filter on Slow Half-Sine Transient input waveform

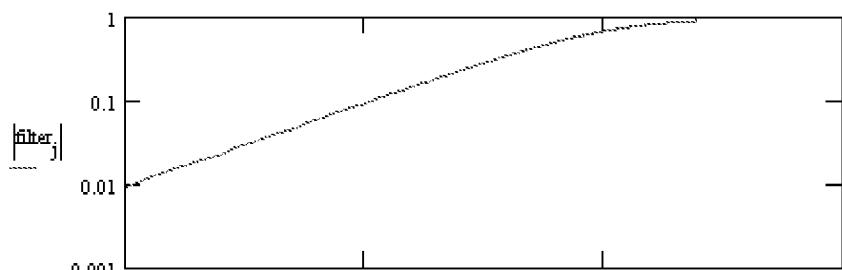
a) Input waveform



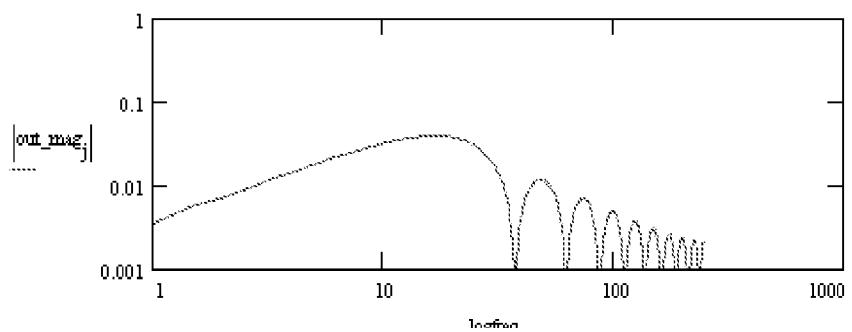
b) Input spectrum



c) Filter characteristic



d) Output spectrum



e) Output waveform

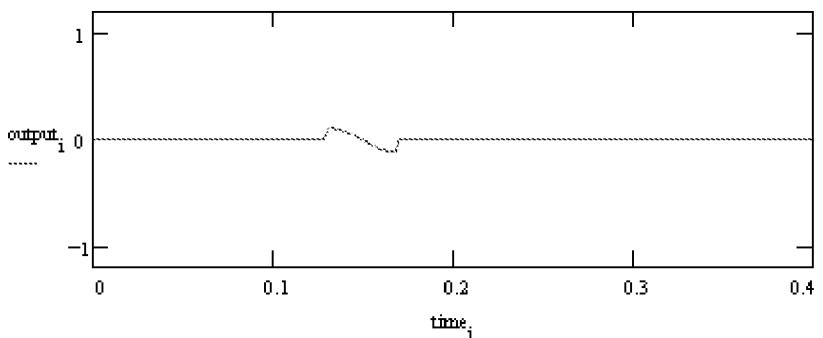
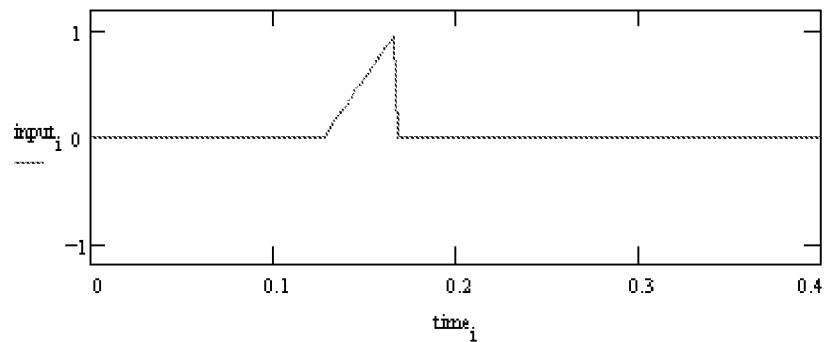
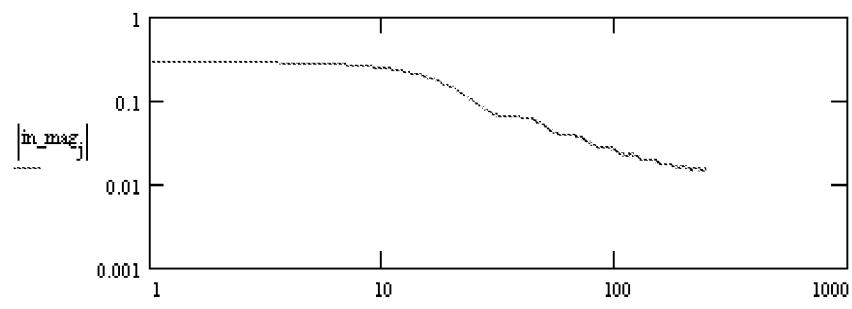


Figure 22. Effect of R-C filter on Slow Sawtooth Transient input waveform

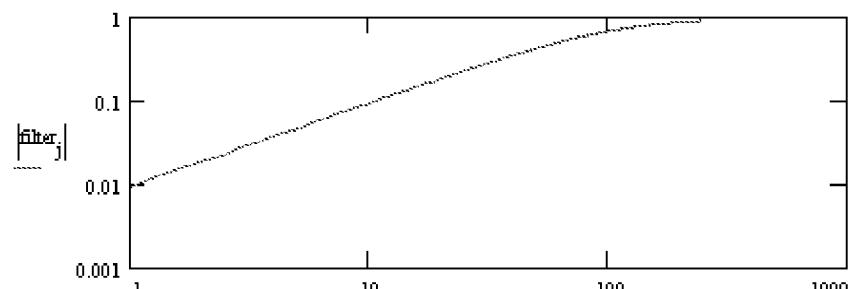
a) Input waveform



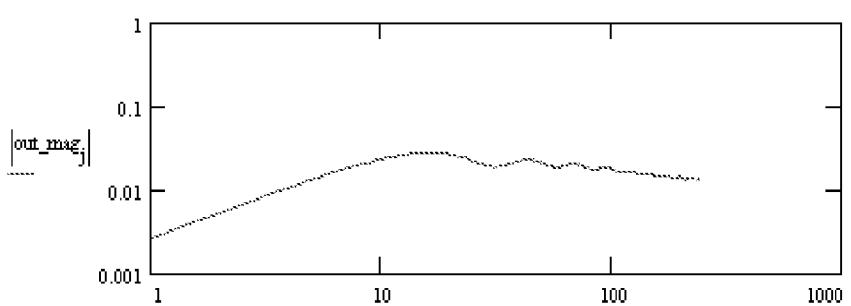
b) Input spectrum



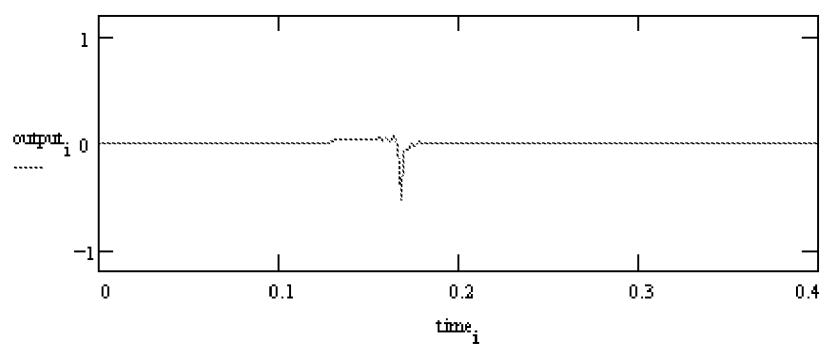
c) Filter characteristic



d) Output spectrum



e) Output waveform

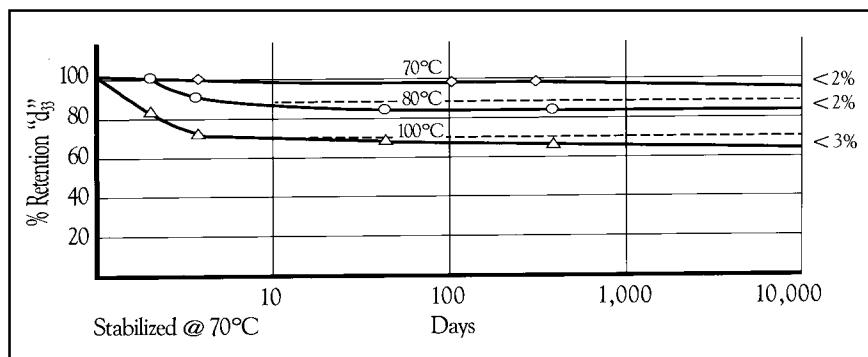


TEMPERATURE EFFECTS

Figure 23. Thermal stability of d_{33} constant - 70°C annealed PVDF

Many of the properties of piezo film change with excitation frequency and temperature. These properties are reversible and repeatable with either frequency or temperature cycling.

In addition, Figure 23 shows the permanent decay of the piezoelectric strain constant d_{33} for PVDF, annealed at 70°C, after long term exposure to elevated temperatures.



Having reached a stabilizing temperature, the material properties then remain constant with time. Piezo film can be annealed to specific operating (or maximum storage) temperatures to achieve long-term stability for high temperature applications. Figure 24 shows the reversible temperature effects on d_{33} and g_{31} coefficients for PVDF.

In Figures 25a and 25b, the effect of temperature on the dielectric constant (ϵ/ϵ_0) and dissipation factor ($\tan \delta_e$) are shown for copolymer films.

Piezo films have been shown to offer excellent transducer properties at very low (cryogenic)

Figure 24. Temperature coefficient for d_{33} and g_{31} constants - PVDF

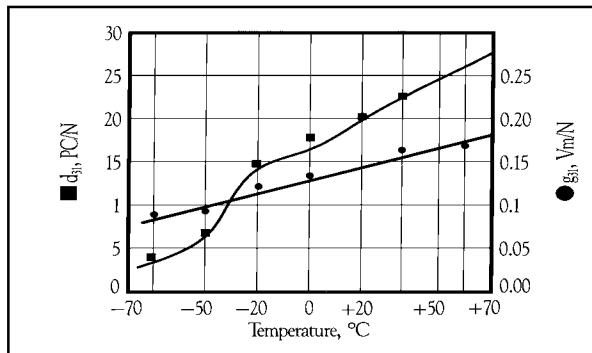


Figure 25a. Dielectric loss tangent vs. temperature

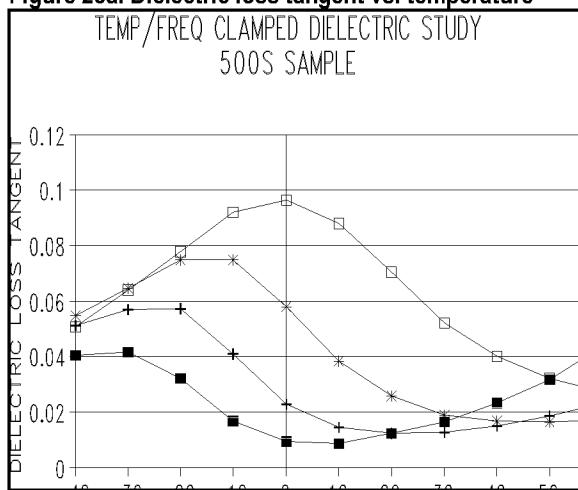
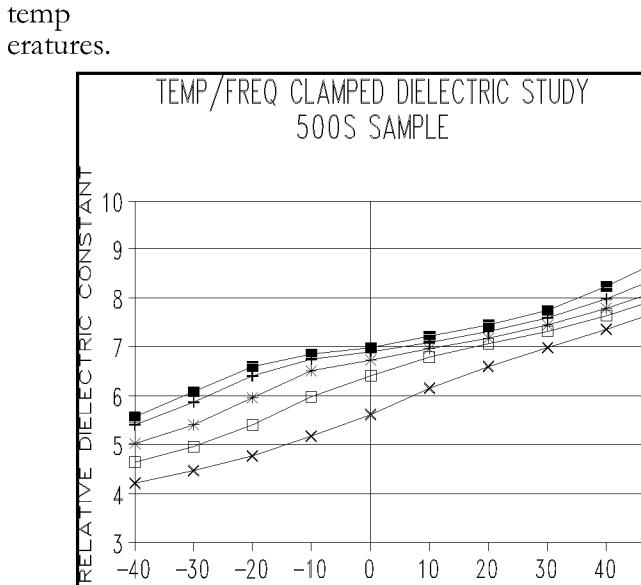


Figure 25b. Dielectric constant vs. temperature COPOLYMER



PIEZOELECTRIC CABLE AND PROPERTIES

One of the most recent developments in piezo polymer technology is piezo cable. The cable has the appearance of standard coaxial cable, but is constructed with a piezoelectric polymer insulator between the copper braid outer shield and the inner conductor (Figure 26).

Protected by a rugged polyethylene jacket, the cable is used in buried or fence security systems, traffic sensors including vehicle classification and weight-in-motion systems, and taxiway sensors for aircraft identification, safety and security applications.

Other applications include sensors for anti-tampering, door edge safety monitoring, floor mats, touch pads and panels, and patient mattress monitors. The new cables feature the same piezoelectric properties that are characteristic of piezo film sensors. The electrical output is proportional to the stress imparted to the cable. The long, thin piezoelectric insulating layer provides a relatively low output impedance (600 pF/m), unusual for a piezoelectric device. The dynamic range of the cable is substantial (>200 dB), sensing distant, small amplitude vibrations caused by rain or hail, yet responding linearly to the impacts of heavy trucks. The cables have withstood pressures of 100 MPa. The typical operating temperature range is -40 to +125 °C. Table 4 lists typical properties for piezo cable.

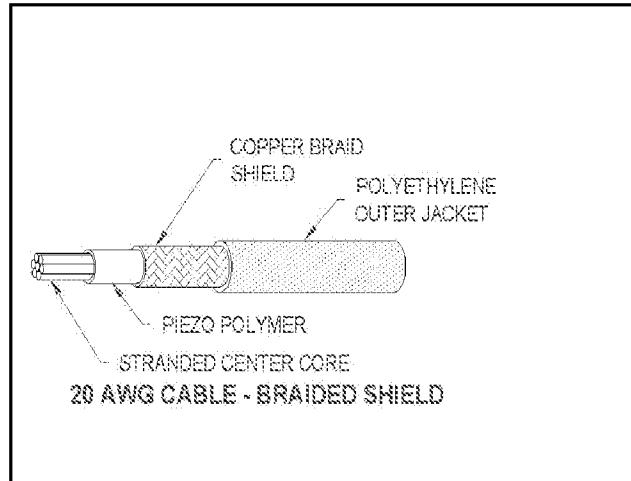


Table 4. Piezo Cable Typical Properties

Parameter	Units	Value
Capacitance @ 1KHz	pF/m	600
Tensile Strength	MPa	60
Young's Modulus	GPa	2.3
Density	kg/m ³	1890
Acoustic Impedance	MRayl	4.0
Relative Permittivity	@1KHz	9
$\tan \delta_e$	@1KHz	0.017
Hydrostatic Piezo Coefficient	pC/N	15
Longitudinal Piezo Coefficient	Vm/N	250×10^{-3}
Hydrostatic Piezo Coefficient	Vm/N	150×10^{-3}
Electromechanical Coupling	%	20
Energy Output	mJ/Strain (%)	10
Voltage Output	kV/Strain (%)	5

Cable Typical Properties

The output sensitivity of piezo cable in response to increasing impact load is shown in Figure 27a. The linearity in output for increasing force as shown in Figure 27b is typical of all piezo cable gages.

PIEZOELECTRIC BASICS

Figure 27a. Sensitivity vs. load

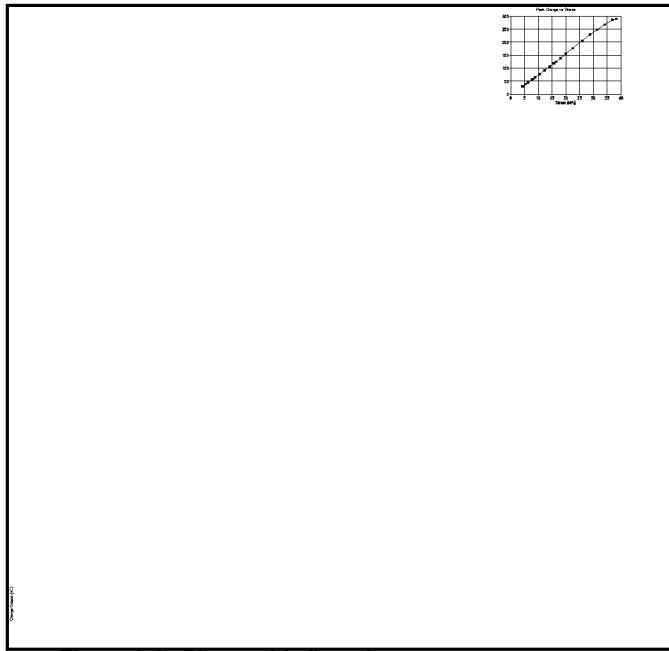
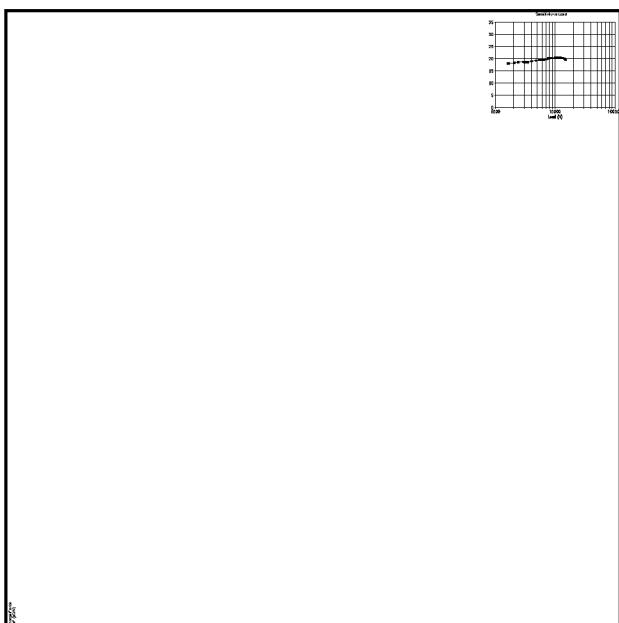


Figure 27b. Piezo cable linearity

Mechanical to Electrical

Like water from a sponge, piezoelectric materials generate charge when squeezed. The amplitude and frequency of the signal is directly proportional to the mechanical deformation of the piezoelectric material. The resulting deformation causes a change in the surface charge density of the material so that a voltage appears between the electroded surfaces. When the force is reversed, the output voltage is of opposite polarity. A reciprocating force thus results in an alternating output voltage.

Piezo film, like all piezoelectric materials, is a dynamic material that develops an electrical charge proportional to a change in mechanical stress. Piezoelectric materials are not suitable static measurements (true dc) due to their internal resistance. The electrical charges developed by piezo film decay with a time constant that is determined by the dielectric constant and the internal resistance of the film, as well as the input impedance of the interface electronics to which the film is connected. Practically speaking, the lowest frequency measurable with piezo film is in the order of 0.001Hz. There are methods to achieve true dc response, but these require using the piezo film as both an actuator and sensor, monitoring change in the actuation resulting from the dc event.

The fundamental piezoelectric coefficients for charge or voltage predict, for small stress (or strain) levels, the charge density (charge per unit area) or voltage field (voltage per unit thickness) developed by the piezo polymer.

Charge Mode:

Under conditions approaching a short circuit, the generated charge density is given by:

$$D = Q/A = d_{3n}X_n \quad (n = 1, 2, \text{ or } 3)$$

The mechanical axis (n) of the applied stress (or strain), by convention, is:

1 = length (or stretch) direction

2 = width (or transverse) direction

3 = thickness direction

where

D = charge density developed

Q = charge developed

A = conductive electrode area

d_{3n} = appropriate piezoelectric coefficient for the axis of applied stress or strain

n = axis of applied stress or strain

X_n = stress applied in the relevant direction

It is important to note that the d_{3n} coefficient is commonly expressed in pico-Coulombs per Newton (pC/N), but the more correct form would be (pC/m²)/(N/m²) since the areas (m²) upon which the stresses or strains apply are very often different and cannot be "canceled".

Voltage Mode:

The open-circuit output voltage is given by:

$$V_o = g_{3n}X_n t \quad (n = 1, 2, \text{ or } 3, \text{ as above})$$

where

g = appropriate piezoelectric coefficient for the axis of applied stress or strain

X_n = applied stress in the relevant direction

t = the film thickness

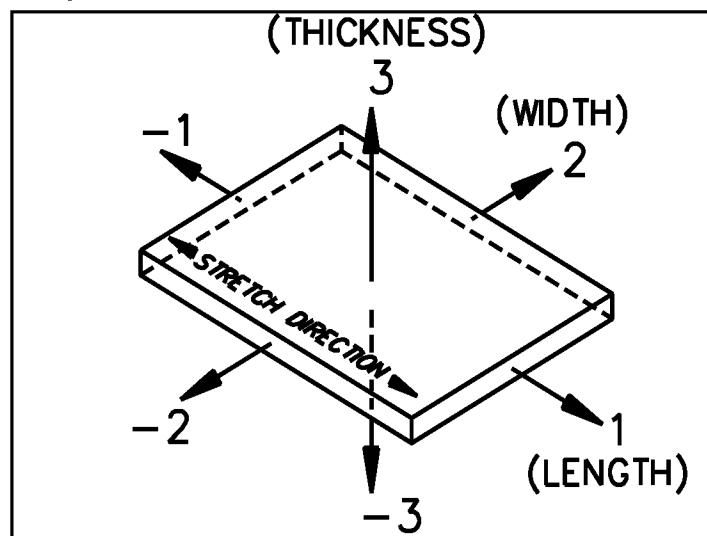
Piezo Coefficients:

The most widely used piezo coefficients, d_{3n} and g_{3n} , charge and voltage respectively, possess two subscripts. The first refers to the electrical axis, while the second subscript refers to the mechanical axis. Because piezo film is thin, the electrodes are only applied to the top and bottom film surfaces.

Accordingly, the electrical axis is always "3", as the charge or voltage is always transferred through the thickness (n = 3) of the film. The mechanical axis can be either 1, 2, or 3, since the stress can be applied to any of these axes, as shown in Figure 28.

Typically, piezo film is used in the mechanical 1 direction for low frequency sensing and actuation (< 100KHz) and in the mechanical 3 direction for high ultrasound sensing and actuation (> 100KHz).

Figure 28. Numerical classification of axes



Directionality:

Piezoelectric materials are anisotropic. This means that their electrical and mechanical responses differ depending upon the axis of applied electrical field or axis of mechanical stress or strain. Calculations involving piezo activity must account for this directionality.

EXAMPLE 1:

A 1.45 psi load ($10,000 \text{ N/m}^2$) is applied to a piezo film switch of 2.54 cm length, 2.54 cm width and $110\mu\text{m}$ in film thickness. The switch element is rigidly backed, so the force acts to compress

```
func {horz 1000 LINESPACE 125 STACKALIGN {V_o`=&`-g_33`Xt # g_33`=&`-339`x`10^`{-3}`{V/m} over {N/m^2}}}
```

the film's thickness (therefore g_{33} mode). In this example the load acts on the length by width area of the piezo film. The open circuit voltage developed across the thickness of the piezo film is: where:

V/m is Volts out per meter of piezo film thickness

N/m^2 is stress applied to the relevant film area. The conversion from psi to

```
func {horz 1000 LINESPACE 125 STACKALIGN {V_o`=&`-` left (-339`x`10^`{-3}`{V/m} over {N/m^2}) right )`(-10,000`N/m^2)(110`x`10^`{-6}`m) # VERT 3 V_o`=&`-0.373`\volts}}
```

N/m^2 is approximately 7,000.

EXAMPLE 2:

The same piezo film element as in EXAMPLE 1 is subjected to a force ($10,000 \text{ N/m}^2 \times 0.0254\text{m}^2 = 6.45$ Newtons), but in this example, the film switch is configured as a membrane having a

```
func {horz 1000 linespace 125
stackalign {V_o`=&`-(3g_31`left ( {F} over {wt}right )`)(t)`=-
(g_31)
left({F} over {w} right) # g_31`=&`216`x`10^`{-3}`{V/m} over
{N/m^2} # V_o`=&`-
left(216`x`10^`{-3}`{V/m} over {N/m^2} right )left ({6.45`N} over
{2.54`x`10^`{-2}`m} right )#
V_o`=&`-54.9`\volts}
```

compliant backing. Now, the force acts on the thickness cross-sectional area (wt). The piezo film is being stretched by the load, so it is acting in the g_{31} mode.

The sharp increase in output voltage results because the force is applied to the much smaller cross-sectional area of the film. The small area results in a correspondingly higher stress.

Dynamic Range

Piezo film has a vast dynamic range. The sensor has been used to detect the impact of high speed particles in space having a mass of 10^{-12} grams, and at the other extreme, measures shock waves at 300,000 atmospheres produced during weapons testing. A recent study was conducted to determine the maximum output energy of a 52 μm thick film, having an area of 155.5 mm x 18.5 mm. The film was subjected to approximately 350 MPa (in the stretch or "n = 1" direction) without failure. The charge generated was found to be very linear, with the following measurements made at maximum applied stress:

Maximum Charge Observed: $20\mu\text{C}$, giving 6.95 mC/m^2
Maximum Voltage Observed: 1600 V, giving $30.8 \times 10^6 \text{ V/m}$
Maximum Energy Converted: 30.9 mJ, giving 207 kJ/m^3

Later experiments showed that about 10% of the above energy levels can be sustained for long periods of time without measurable damage to the piezo film element.

Electrical to Mechanical

When a voltage is applied to a sheet of piezo film, it causes the film to change dimensions due to the attraction or repulsion of internal dipoles to the applied field. With one voltage polarity is applied, the piezo film becomes thinner, longer and wider. The opposite polarity causes the film to contract in length and width and become thicker. An ac voltage causes the film to "vibrate".

The amount of deformation is given by the piezoelectric "d_{3n}" constant:

for **length** change $\Delta l \sim$

where

Δl = change in film length in meters
 l = original film length in meters
 d_{31} = piezoelectric coefficient for length ("n=1" direction) change in meters per volt
 V = applied voltage across the thickness (t)

for **width** change $\Delta w \sim$

where

d_{32} = piezoelectric coefficient for width ("n=2" direction) change

for **thickness** change $\Delta t \sim$

where

d_{33} = piezoelectric coefficient for thickness ("n=3" direction) change

EXAMPLE 3:

A piezo film of 3 cm length (l), 2 cm width (w) and $9\mu\text{m}$ thickness (t) is subjected to an applied voltage of $V=200$ volts in the z (thickness) direction. The amount of strain S resulting from this electrical input is d times the applied field.

```
func {horz 1000 linespace 150 stackalign {S_1``=&``{DELTA`l`}} over {l}```=``d_31`{V/t)``\n`where~d_31``=``23`x`10^{\-12}`{m/m} over {V/m} # Horz 1 DELTA`l`=&``d_31(V/t)`l`=\``\left(23`x`10^{\-12}`{m/m} over {V/m} right) {(200`V)`(3`x`10^{\-2}`m)} over {(9`x`10^{\-6}`m)} #\nHorz 1 DELTA`l`=&``1.53`x`10^{\-5}`m``\or``15.3 ~mu m`}
```

In the l direction:

```
func {horz 1000 DELTA`l`="`td_33(V/t)`l`="`d_33 V = left(-33`x`10^{\-12}`{m/m} over {V/m}\nright ) `left ( 200`V right ) ``=`` 6.6`x`10^{\-9}`m ``\or``66\AA` }
```

In the t direction:

Actuators

Generally, piezo film actuator designs depend on the application requirements such as operating speed, displacement, generated force, and available electrical power. Piezo film technology offers various design options to meet such application requirements. Those design options include:

- Customized electrode patterns on one or both sides of the piezo film sheet.
- Multilaminate structures or bimorphs.
- Fold-over or scrolled multilayer structures.
- Extruded piezo tubes and piezo cables.
- Cast piezo polymer on various substrates
- Molded 3-D structures.

Each design option mentioned above has advantages and disadvantages. For example, scrolled multilayer actuators can generate a higher force but may sacrifice some displacement.

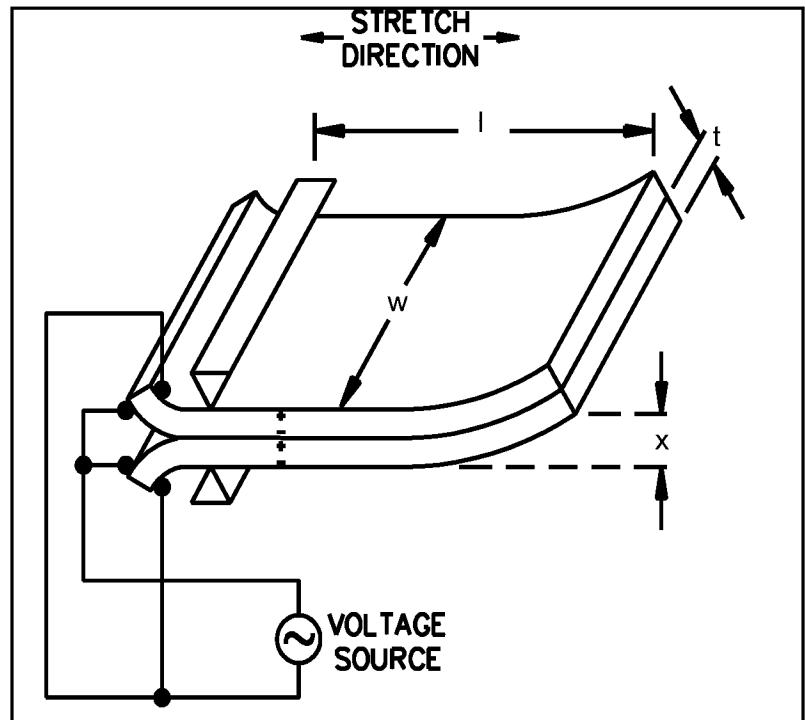


Figure 29. Piezo film bimorph

Bimorph

Like a bimetal strip, two sheets of piezo film of opposite polarities, adhered together form a bending element, or "bimorph" (Figure 29). An applied voltage causes one film to lengthen, while the other contracts, causing the unit to bend. An applied voltage of opposite polarity bends the bimorph in the opposite direction.

The bimorph configuration converts small length changes into sizable tip deflections, but producing low force.

Thicker films and multilayers improve the force developed by the bimorph, but sacrifice displacement unless the unit can be operated at higher fields.

The amount of tip deflection and the force developed are given by:

$$\Delta x = \frac{3}{4} V d_{31} l^2 = \text{meters}$$

and

$$F = \frac{3}{4} V d_{31} l^2 E = \text{Newtons}$$

where

Δx = displacement at dc

F = generated force

d_{31} = piezoelectric coefficient in the "1" direction

l, t, w = length, thickness, and width of piezo film

V = applied voltage (Volts)

Y = Young's modulus of piezo film ($2 \times 10^9 \text{ N/m}^2$)

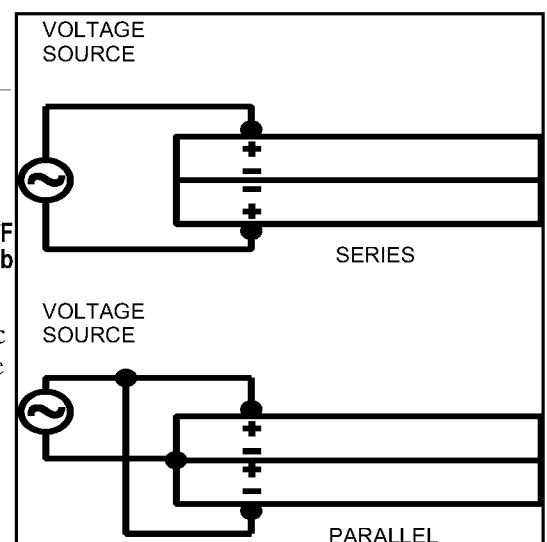
By applying an ac voltage, the bimorph can act as a fan, similar to an insect wing. Although the piezo film bimorph does exhibit a dc response, maximum tip deflections are obtained when the unit is operated at resonance, determined by the length and thickness of the bimorph beam.

EXAMPLE 4:

```
func {horz 1000 linespace 150 stackalign {DELTA x``=&``{3/4Vd_31`l^2} over
{t^2} # horz 1
DELTA x``=&``{{3/4(100`V) left(23`x`10^{-12}`{{m/m} over {V/m}}}
right)`(2`x`10^{-2}`m)^2} over {(9`x`10^{-6}`m)^2}} # horz 1
DELTA x``=&``8.52 mm}}
```

100 volts are applied across a 2 cm long cantilever bimorph comprised of two strips of 9 PVDF. The resultant tip displacement is:

As shown in the equations, more displacement can be obtained from a longer bimorph. Larger forces can be obtained from a wider bimorph. The ratio of displacement at a resonance frequency and dc is defined by Q which indicates a mechanical gain. A typical Q value for a piezo film bimorph is 20 to 25.



For example, a 5 mm long $70\mu\text{m}$ thick bimorph with 120 volts dc creates a displacement of $57\mu\text{m}$. With the same bimorph, however, displacement can be 1.4 mm at the resonant frequency of 580 Hz. For applications that require a higher force, such as cooling fans, multilayer construction can be considered. The resulting output force is proportionally increased by the number of layers.

In terms of electrical connections to the bimorph, there are two basic methods as shown in Figure 30 — parallel and series connections. In order to generate the same amount of displacement, the parallel connection requires a lower voltage than the series connection. Series connections, on the other hand, draw less current than parallel connections. For both parallel and series connections, the total electrical power to the actuator is identical. However, it is obvious that the lead attachment of the series connection is much simpler than that of the parallel connection for manufacturing purposes. Typical applications of the bimorph bender are cooling fans, toys, and decoratives.

Scrolled Actuator

The generated force and displacement of a scrolled piezoelectric cylinder in Figure 31 are expressed as follows:

```

func {horz 200 x``=``d_{31}El}
func {horz 200
F``=``Yd_31EA}

func {horz 200 f``=``(1/2 pi) SQRT
{Y`A/l`{M_e`+`0.405`M_p}}}

```

Meters

Volts/meters

Newton

Figure 31. Scrolled piezo film actuator

where

x = displacement at dc (meter)

F = generated force (Newton)

f = resonance frequency

l, t = Length, thickness of piezo film (meters)

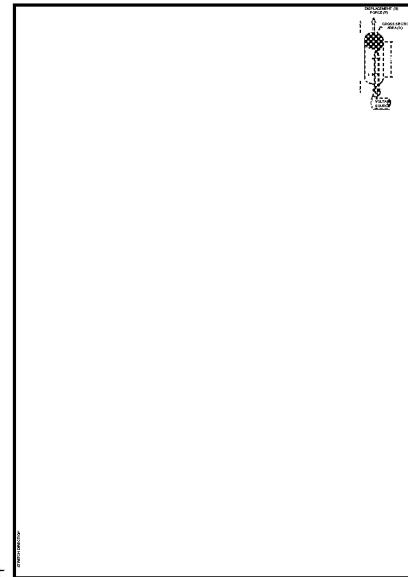
M_e = externally loaded mass (kilograms)

M_p = piezo actuator mass (kilograms)

A = cross sectional area (m^2)

Y = Young's modulus (N/m^2)

E = electrical field (volt/meter)



As shown in the equations, a scrolled actuator can generate more force and can respond with a higher resonant frequency by increasing the cross sectional area. A longer actuator generates more displacement but reduces the response speed. Note that the actuator output, with $M_e = 0$, will be maximized when the length l is adjusted to satisfy the resonant condition. As an example, the performance of a 12 mm diameter, 25 mm long scrolled actuator can be maximized at 32 KHz operation.

Folded Actuator

Another design option for a high speed, high force actuator is to fold over a long sheet of piezo film as shown in Figure 32. This design effectively creates a parallel wired stack of piezo film discs. The center hole is used to secure the actuator to a base. Design equations of the scrolled actuator also can be applied to this type of actuator. In the previous equations, d_{31} should be replaced with d_{33} ($-33 \times 10^{-12} \text{ C/m}^2$) for a folded actuator. An example of specifications for the folded actuator is shown below:

Displacement: $1 \mu\text{m}/1 \text{ mm length}$

Generated force: $15 \text{ kg}/10 \text{ mm dia.}$

Frequency: dc - 100 kHz

Drive voltage: 800 volts

Compared to mechanical or piezo ceramic actuators, multilayer piezo film actuators have fewer ringing problems due to their lower Q. Applications of multilayer actuators are micropositioners for industrial equipment, acoustic wave generators and ink jet printers.

Ultrasonic Actuators

Ultrasonic actuators, as discussed in this section, exclude very high frequency (> 1 MHz) transmitter applications. The use of piezo film in these very high frequency applications, like medical ultrasound imaging and nondestructive testing, use thickness mode operation, d_{33} . This section deals with low frequency ultrasound (20-100 KHz) where the piezo film can be used in the length change (d_{31}) mode.

The advantage of piezo film in low frequency ultrasound can be found from the flexibility of the material. Piezo film can be easily curved or formed to make circular transducers as shown in Figure 33. The beam pattern is determined by the number of half circular elements and their diameter. The operating frequency is determined by the diameter of the half circular elements. Note that the difference between Figures 33(a) and 33(b) is their number of active elements and diameters. To widen the beam coverage, the number of active elements should be reduced. With a cylindrical transducer, a 360° beam pattern is obtained.

In ultrasound applications, a narrow beam with minimum side lobes is required for remote distance measurements. On the other hand, a wide beam, as wide as 180° or more, is required for applications like automobile rear bumper proximity sensing. Figure 33 shows design configurations for both narrow beam and wide beam ultrasound transducers. The applications for piezo film in through-air ultrasonic actuators include distance ranging for air pen, air mouse, white board digitizer, collision avoidance, physical security systems, air flow velocity (doppler) sensors, and inter-object communications. Similar constructions can be produced for underwater or fluid sensing, including flow sensors, level sensors, and communications.

PYROELECTRIC BASICS

Piezoelectric polymers, such as PVDF and its copolymers of VF_2/VF_3 , are also pyroelectric. Pyroelectric sensor materials are normally dielectric materials with a temperature-dependent dipole moment. As these materials absorb thermal energy, they expand or contract, thereby inducing secondary piezoelectric signals. As piezo film is heated, the dipoles within the film exhibit random motion by thermal agitation. This causes a reduction in the average polarization of the film, generating a charge build up on the film surfaces. The output current is proportional to the rate of temperature change (ΔT). The amount of electrical charge

Figure 32. Folded piezo film actuator

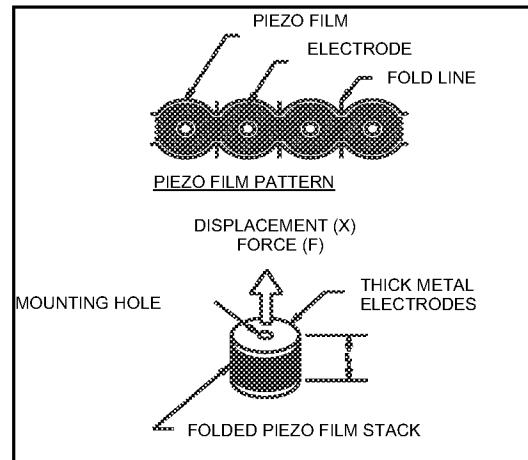
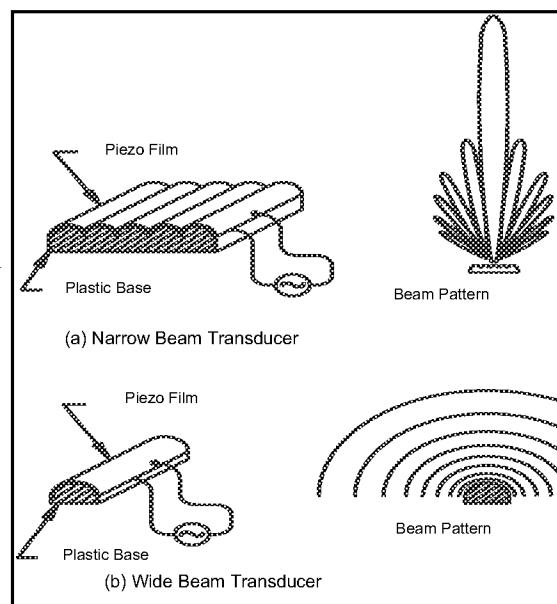


Figure 33. Piezo film ultrasound transducers



produced per degree of temperature increase (or decrease) is described by the pyroelectric charge coefficient, ρ .

The charge and voltage produced in a given film of area A permittivity ϵ , and thickness t is given by:

```
func {linespace 150 stackalign {Q``=&``p DELTA`TA
# V``=&``pt DELTA`T/\epsilon}}
```

EXAMPLE 5:

A piezo film pyroelectric detector having a film thickness (t) of $9\mu\text{m}$, a permittivity (ϵ) of $106 \times 10^{-12} \text{ C/Vm}$ and a pyroelectric coefficient (p) of $30 \times 10^{-6} \text{ C}/(\text{m}^2\text{K})$, undergoes a temperature increase

```
func {horz 1000 linespace 150 stackalign {V``=&``{(30`x`10^{-6}`C/m^2` DEG K)(9`x`10^{-6}`m)(1 DEG K)} over {(106`x`10^{-12}`C/Vm)}
# horz 1 V``=&``2.55~\volts }}
```

(ΔT) of 1°K due to incident IR radiation. The output voltage is given by:

The pyroelectric voltage coefficient of piezo film is about an order of magnitude larger than those of Lead Zirconate Titanate (PZT) and Barium Titanate (BaTiO_3). Table 5 compares the pyroelectric properties of these materials, but a far lower figure of merit due to the low capacitance of PVDF.

Table 5. Comparison of pyroelectric materials

Material	TGS	LiTaO_3	BaTiO_3	PZT	PbTiO_3	PVDF	VF_2VF_3
func {	350	200	400	420	230	30	50
$\epsilon`/\epsilon$	30	45	1000	1600	200	10.7	8.0
α	.16	1.31	1.00	.44	.67	.06	.06
L	225	646	564	374	461	138	138
P_v	1.32	.50	.05	.03	.10	.47	.71
M_l	.53	.16	.02	.01	.03	.20	.31
Pyroelectric Charge Coefficient	$(\rho_Q)\mu\text{Coul}/[\text{m}^2 \cdot {}^\circ\text{K}]$						
Dielectric Constant	func {(ϵ/ϵ_0), where $\sim \epsilon_0 =$						
Thermal Diffusivity	$(\alpha)\text{m}^2/\text{sec} \cdot 10^{-6}$						
Thermal Diffusion Depth @ 1Hz	$(L)\mu\text{m}$						
Pyroelectric Voltage Coefficient	$(P_v)\rho_Q/\epsilon, \text{V}/[\mu\text{m} \cdot {}^\circ\text{K}]$						
Figure of Merit	$(M_l)\rho_Q/[C_v \cdot \epsilon], \text{V} \cdot \text{mm}^2/\text{J}$						

Piezo film advantages including:

- moisture insensitivity (<.02% H₂O absorption)
- low thermal conductivity
- low dielectric constant
- chemical inertness
- large detector sizes

The pyroelectric response of piezo film can also become a noise source for piezo sensor applications at low frequencies. In piezoelectric applications where low frequency strain sensing is desired, there are several convenient methods to “common-mode reject” the pyroelectric response. Examples include:

- Two equal sized electrode patterns on one piezo film element; one electrode oriented parallel to the d₃₁ and the other electrode pattern is perpendicular to the d₃₁ direction. Both develop equal signals in response to pyro, but the electrode area parallel to the d₃₁ develops about 10X the perpendicular electrode pattern. Subtracting the signals yields a pure piezo response.
- Two equal sized piezo film elements, laminated in a stacking configuration; one film has d₃₁ parallel to strain surface, the other has d₃₁ perpendicular to strain surface. As above, signals are subtracted to isolate the piezo response from pyro.
- Several other common mode rejection techniques can be described by MSI's applications engineers.

For higher frequencies, where the rate of temperature change seen by the piezo film element is slower than the strain event to be measured, frequency filters readily sort out the unwanted pyro signal.

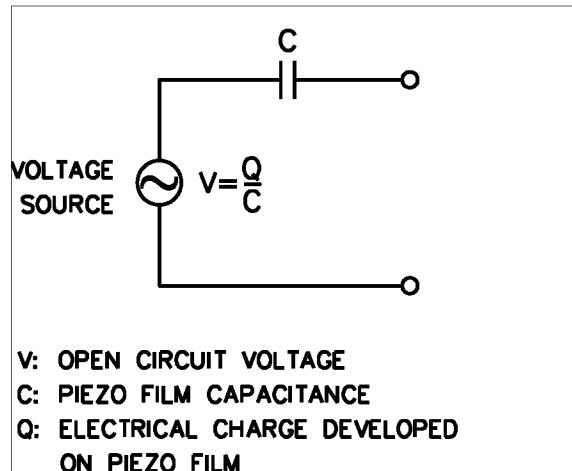
BASIC CIRCUIT CONCEPTS

A properly designed interface circuit plays a key role in the optimization of piezo film sensors. The applications of piezo film span from toys to military sensors and interfacing to electronics is highly application dependent. In many cases, piezo film can be directly connected to electronic circuits without special interface considerations. However, for those cases where an interface circuit is required, the following 3 steps are recommended:

1. Consider the frequency range and signal amplitude requirements over the desired dynamic range.
2. Choose a proper load resistance to assure the low end operating frequency and to minimize signal loss due to the loading effect.
3. Select a buffer circuit if the signal level is small. If a high value load resistance is needed (such as 22MΩ or higher value), a low leakage high impedance buffer amplifier is recommended. JFET's or CMOS operational amplifiers are commercially available for a buffer.

Simplified Equivalent Circuits

Figure 34. Equivalent circuit of piezo film



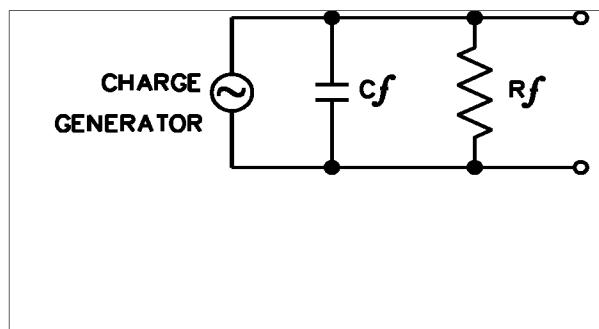
The first step in an interface circuit design is to understand the piezo film characteristics as part of an electrical equivalent circuit. Figure 34 shows a simplified equivalent circuit of piezo film. It consists of a series capacitance with a voltage source. The series capacitance C_f represents piezo film capacitance which is proportional to the film permittivity and area and inversely proportional to film thickness. The voltage source amplitude is equal to the open circuit voltage of piezo film and varies from microvolts to 100's of volts, depending on the excitation magnitude. This simplified equivalent circuit is suitable for most applications but is of limited value at very high frequencies such as that used in ultrasound transducers.

Figure 35 shows an equivalent circuit as a charge generator.

This equivalent circuit has film capacitance C_f , and internal film resistance R_f .

The induced charge Q is linearly proportional to the applied force as described earlier. The capacitance C_f is proportional to the surface area of film and is inversely proportional to the film thickness. In low frequency applications, the internal film resistance R_f is very high and can be ignored. The open circuit output voltage can be found from the film capacitance; i.e., $V = Q/C_f$.

Figure 35. Equivalent circuit for piezo film



Input Resistance

The most critical part of an interface circuit is the input resistance. The input resistance affects low frequency measurement capability as well as signal amplitude. This is called the "loading effect".

Piezo film capacitance can be regarded as an equivalent source impedance. It is important to note that this source

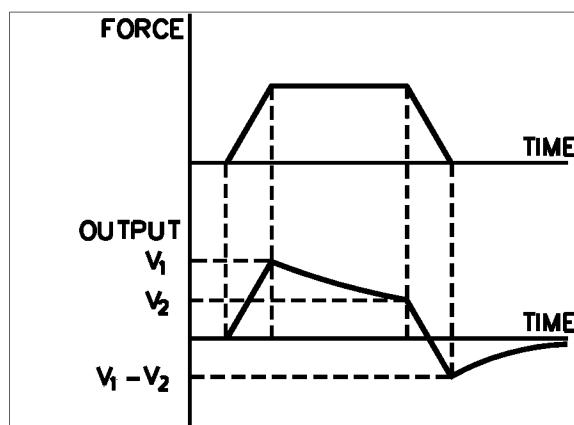
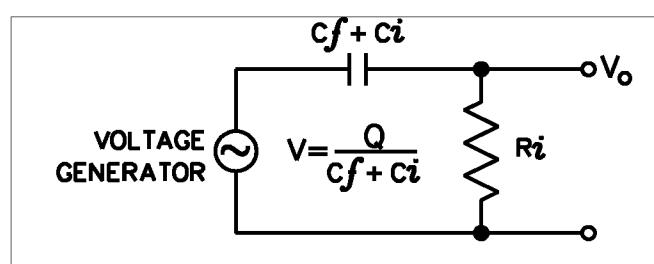


Figure 36. Equivalent circuit of piezo film with input resistance of electronic interface



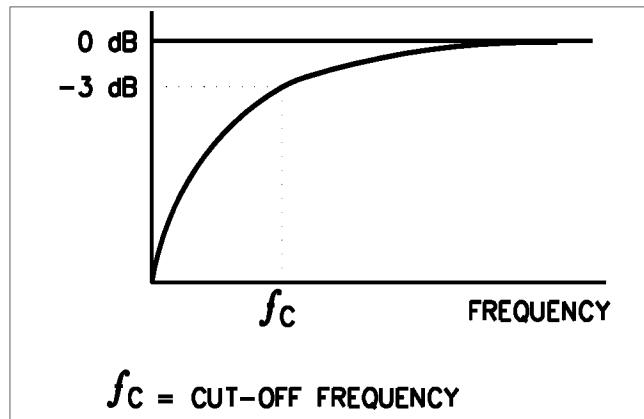
impedance increases with decreasing film capacitance and decreasing frequency of operation. This source impedance

combined with the input resistance produces a voltage divider. As the ratio of input resistance to source impedance is decreased, the overall output voltage is reduced.

Therefore, choosing a proper input resistance for the electronic interface is critical in minimizing the loading effect.

Time Constant

In addition to input resistance, the input capacitance of an interface circuit can also affect the output. Figure 36 shows the equivalent circuit of film with input resistance R_i and input capacitance C_i . A typical time domain response of piezo film is shown in Figure 37. The charge developed on the film due to an applied force decays with a time constant which is defined by $R_i(C_f + C_i)$.



This time constant represents the time required for a signal to decay to 70.7% (-3dB) of its original amplitude. The smaller the time constant, the quicker the signal decays. Because of this finite time constant, piezo film is suitable for dynamic measurements rather than static measurement (0.001 Hz minimum).

If a long time constant is desired, a high input resistance and film capacitance can be used. It should be understood, however, that a high input resistance can also produce higher noise, requiring compensation through shielding, etc.

Frequency Response

Figure 38. High pass filter characteristic of piezo film

Another important aspect of the time constant can be seen in the frequency response of the equivalent circuit. The circuit exhibits an RC high-pass filter characteristic as shown in Figure 38. In this figure, the vertical axis implies the ratio of observable output signal to the developed ~~signal~~ circuit voltage of the piezo film. Zero dB implies no loss of signal. The cutoff frequency (3 dB down) is inversely proportional to the time constant. When a piezo film sensor is operated below this cut-off frequency, the output signal is significantly reduced. For a low frequency measurement, an input resistance needs to be high enough so that the cut-off frequency is well below the desired operating frequency. This consequence can be verified from consideration of the time constant as well as the loading effect.

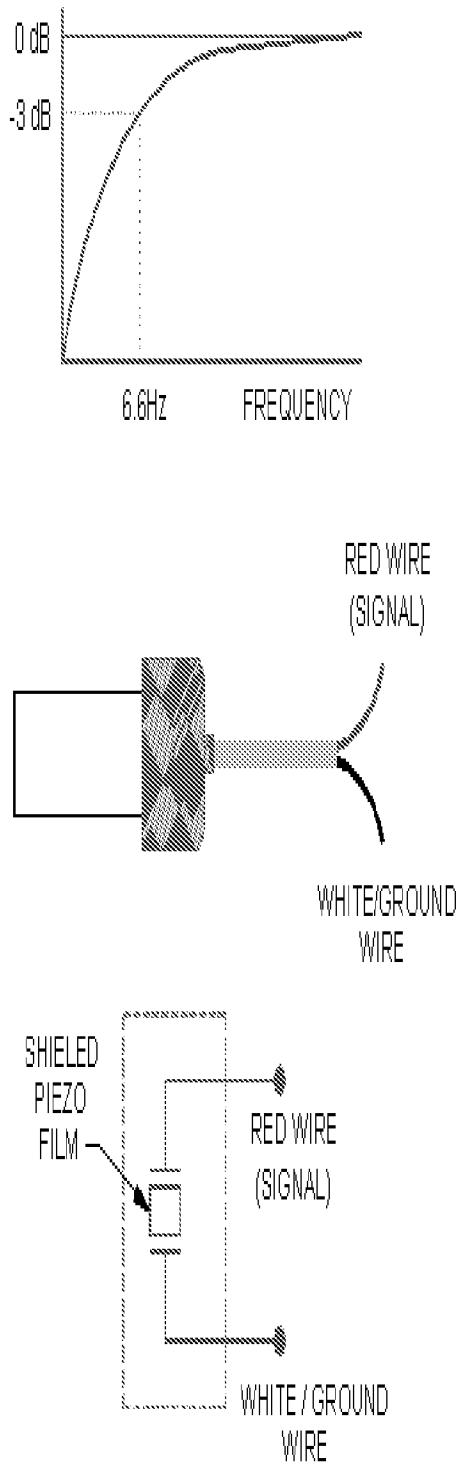
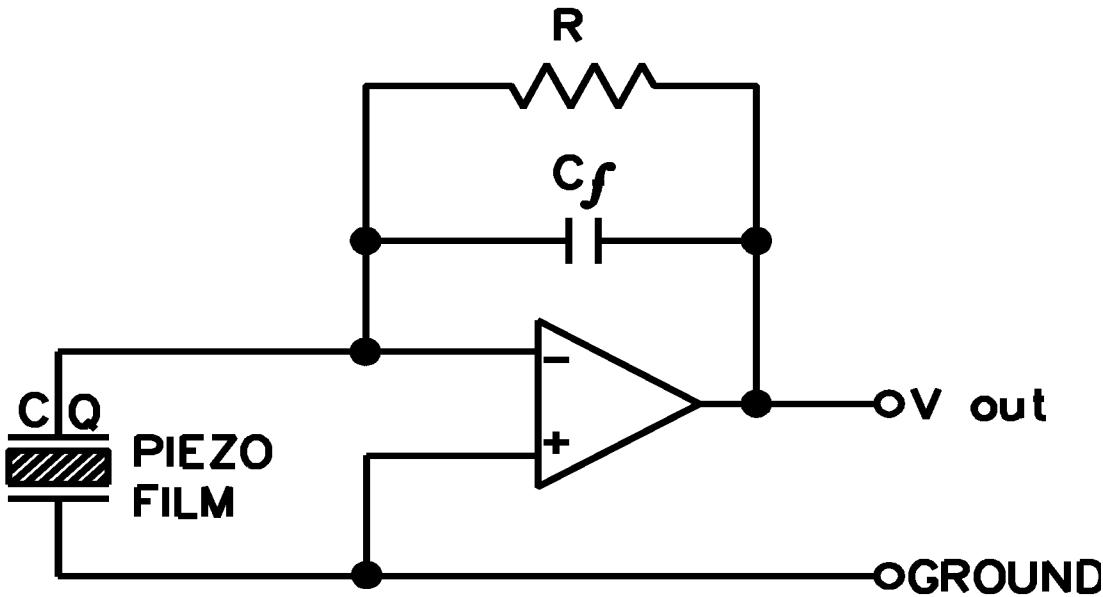


Figure 41. Typical amplifiers for piezo film sensors

(a) A CHARGE AMPLIFIER



C_f = FEED BACK CAPACITANCE

As an example, the frequency response of a shielded piezo film sensor (model SDT1) is shown in Figure 39. In this example, the SDT is interfaced with a circuit which contains a $10M\Omega$ load resistor and an FET. The capacitance of the piezo film is 2.4 nF. With $10M\Omega$ load resistance, the time constant becomes 24 msec and thus, the cut-off frequency is 6.6 Hz. For comparison, the cut-off frequency can be reduced to 0.66 Hz if a $100M\Omega$ resistor is used instead of the $10M\Omega$ resistor. This sensor component can be used for any application operating above the cut-off frequency determined by the resistance value.

In applications where the electronic circuit cannot be placed near the sensor, a buffer circuit is recommended close to the sensor. The buffer circuit converts the high output impedance of the piezo film element into a low output impedance and thus minimizes the signal loss and noise through the cable. For large size (i.e., high capacitance) piezo film sensors a buffer may not be required, even with small signals and long cables.

Figure 40. Unity gain buffer for piezo film sensors

When a high piezo film output impedance is required, a low-leakage, high impedance buffer is necessary. For example, infrared motion sensor and accelerometer applications require up to $50G\Omega$ of input resistance to obtain a very low frequency response. For such cases, the input impedance of the buffer must be much higher than the output resistance of the piezo film in order to maintain the low frequency response. In addition, minimum leakage current of the buffer is critical in order to maximize the measurement accuracy. Some examples of low leakage buffer electronics include: JFET - 4117 (Siliconix, Sprague); Operational amplifiers — LMC660, LF353 (National Semiconductor), OP80 (PMI), and 2201 (Texas Instruments).

Figure 40 shows unity gain buffer circuit examples for general applications. Operational amplifiers offer a great deal of versatility as both buffers and amplifiers. They can be used as either charge-mode or voltage-mode amplifiers. Figure 41 shows basic charge and voltage amplifier configurations. The voltage output of the charge

amplifier is determined by Q/C_f . Q is the charge developed on the piezo film and C_f is the feedback capacitance of the charge amplifier.

The output voltage of the charge amplifier depends on the feedback capacitance, not the input capacitance. This indicates that the output voltage of a charge amplifier is independent of the cable capacitance. The major advantage of a charge amplifier can be realized when a long cable is used between a piezo film sensor and electronics. In addition, it also minimizes charge leakage through the stray capacitance around the sensor. Otherwise, simple voltage amplifiers are sufficient for most applications. Included in Figure 41 is a typical non-inverting voltage amplifier.

The advantage of a voltage amplifier can be seen when ambient temperature is considered. The voltage sensitivity (g-constant) variation over temperature is smaller than the charge sensitivity (d-constant) variation. Consequently, voltage amplifiers with piezo film exhibit less temperature dependence. In Figure 41, the time constants for the charge amplifier and voltage amplifier are determined by RC_f and RC respectively.

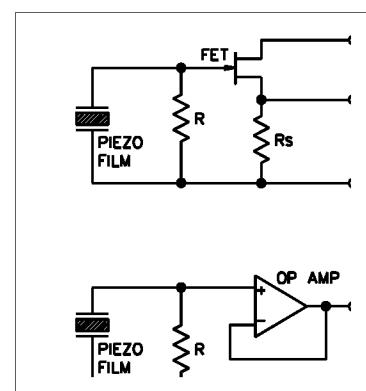
As a design example, a traffic sensor interface is described. Because of its flexibility, piezo cable is an ideal sensor material for traffic measurement applications. MSI's BL traffic sensor is constructed with a piezo cable sheathed in a compressed brass tube, with a variety of signal cable lengths tailored to the installation requirements. The BL is available in sensing lengths of more than 3 meters. In this specific example, the BL sensor is 2 meters long. This electrically shielded sensor has 100 feet of coax cable. The electrical specifications of this sensor include:

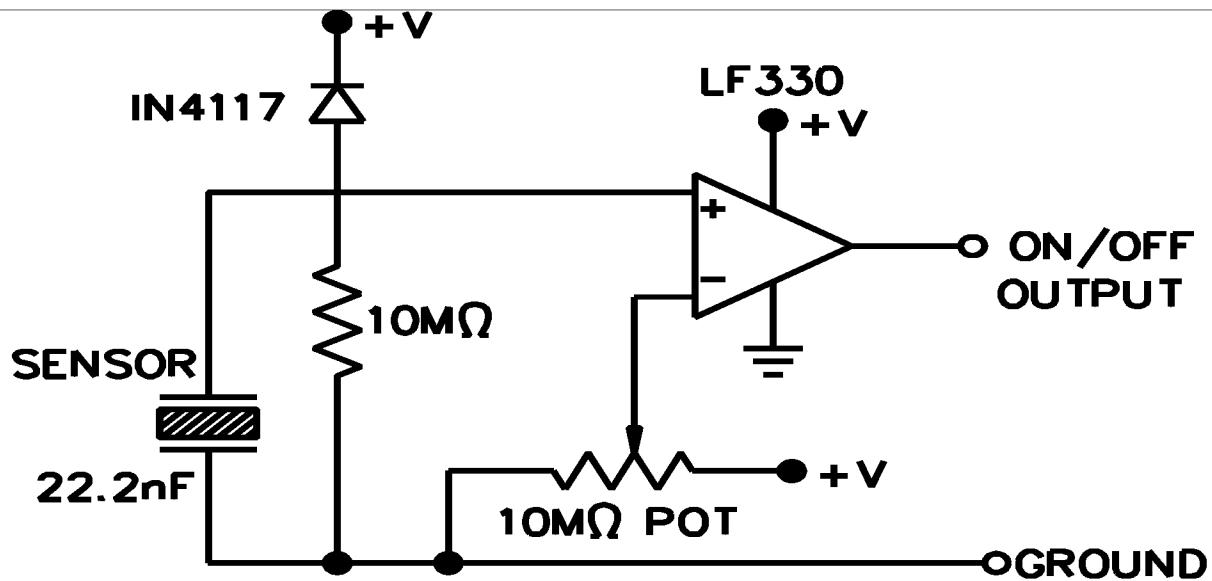
Capacitance = 9.5 nF (including piezo cable and signal cable capacitances)
 Output = 500mV (for a wheel load of 800 pounds at 55mph and 70°F)
 Signal : Noise = 10:1

The basic requirements of an interface circuit are:

Low end frequency = 1.6 Hz
 Circuit output = Digital pulse count

An interface circuit to meet these requirements is shown in Figure 42. This circuit works as a comparator. A $10M\Omega$ input resistance is chosen in order to reduce the cut-off frequency to about 1 Hz. The actual cut-off frequency with this resistor can be calculated as 1.6 Hz. A $10M\Omega$ potentiometer is used to adjust the threshold voltage, V and the diode is included to protect the electronics from high voltage damage. Typical piezo film and interface circuit output signals from a passenger car at 55 mph are shown in Figure 42.





SENSOR CAPACITANCE = 22.2nF
CUT-OFF FREQUENCY = 0.7Hz

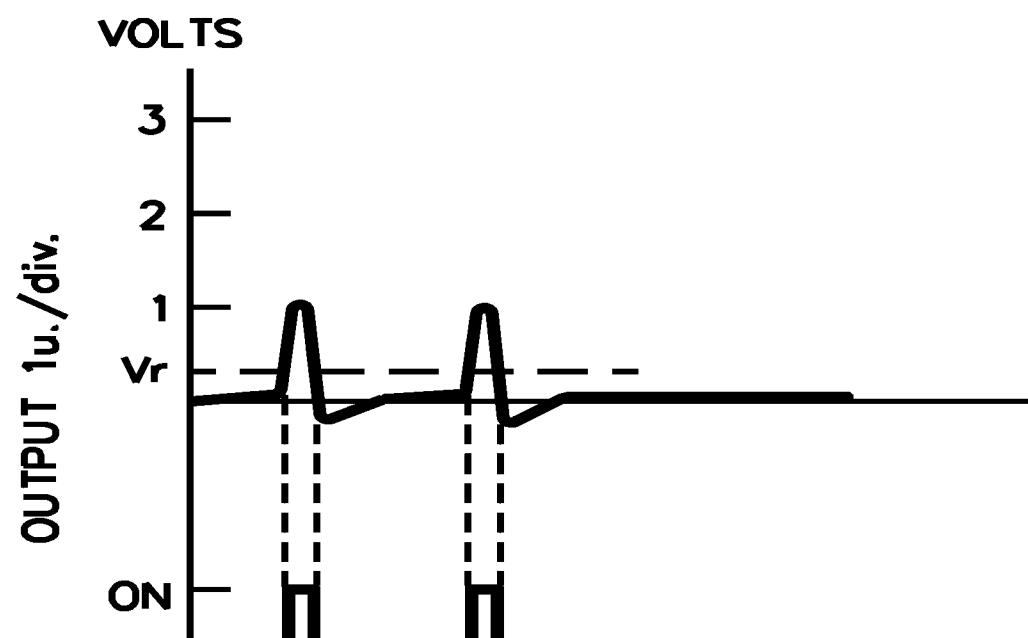


Figure 43. High frequency, low gain FET circuit interface

Signal Conditioning

Because piezo film is both piezoelectric and pyroelectric, some provision must be made to eliminate—or at least reduce—the effect of unwanted signals. The primary principles of signal conditioning include:

- Filtering—Electrical filters designed to give the desired band-pass and band-rejection characteristics.
- Averaging—if the desired signal exhibits periodicity, while the undesired signal is random, signal averaging can increase the signal-to-noise ratio.
- Common Mode Rejection—By wiring two equal areas of a piezo film electrode out-of-phase, unwanted common-mode signals can be made to cancel.

Basic Switch Circuitry

A variety of circuits are available to electronically interface with piezo film including field effect transistors (FETs), operational amplifiers (Op Amps), and low-current digital logic (CMOS).

FETs lend themselves to applications of small size since they are readily available in surface mount technology. Important characteristics to consider when using FETs are switching frequency, piezo film capacitance, leakage current of the FET in the off-state, input bias resistance, and shielding from electromagnetic interference (EMI).

Figures 43 and 44 show typical FET circuit configurations for a piezo film switch. Figure 43, the common drain or source follower, applies well in applications where simple buffering is important. Here, the circuit voltage gain is approximately one.

The common source circuit in Figure 44 is suitable for low frequency applications where voltage gain is required. The gain is determined by resistances R_D and R_S . As the gain increases, frequency bandwidth decreases by a factor of one decade per 20 dB of gain.

Operational amplifiers offer a great deal of versatility for piezo film switch applications. Adaptation to a particular application is often as simple as making a few wiring changes. Important op amp circuit characteristics include input bias resistance, film switch capacitance, and EMI shielding.

The op amp circuit of Figure 45, a charge amplifier, suits applications where a detected vibration actuates the switch. It also works well in small signal applications. A charge amplifier eliminates the effects of the time constants of both the piezo film and

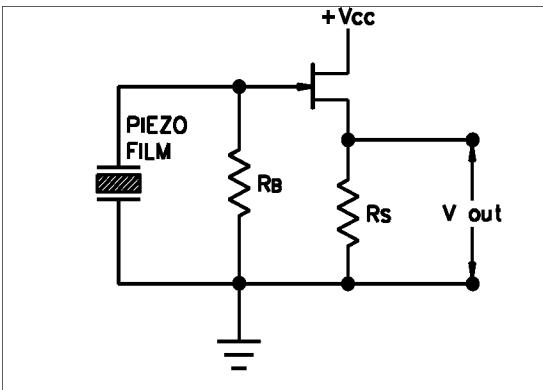


Figure 44. Low frequency, high gain FET circuit interface

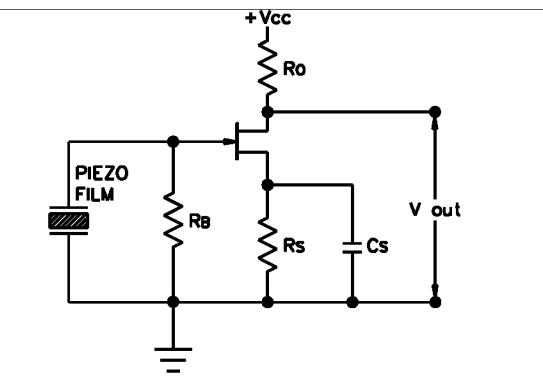
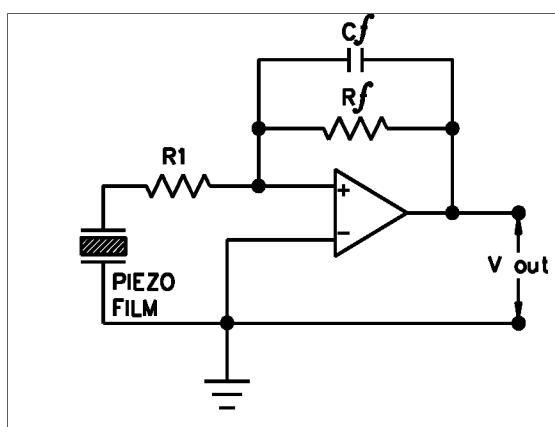
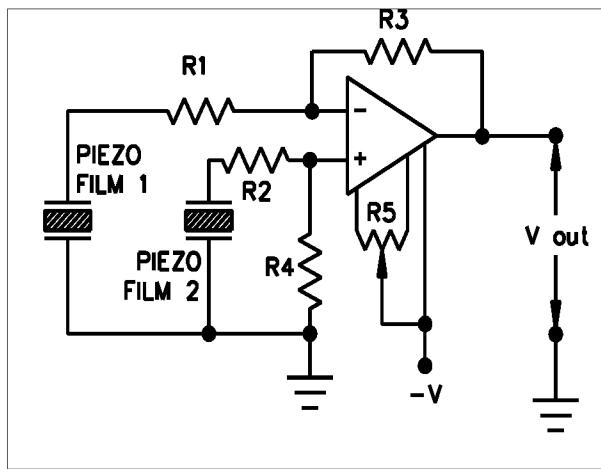


Figure 45. Op Amp Interface circuit acting as a charge amplifier



connecting cable. The charge amplifier is a current operated circuit with zero input impedance, which

Figure 48. Differential Op Amp interface circuit



results in no voltage being generated across the film. The charge amplifier quickly absorbs charges developed by the film.

With no charge left on its electrodes, the film exhibits no time constant.

The capacitance of the film and connecting cable have no adverse effect on the circuit's transfer function. Thus tolerances on film

size and cable length need not be exceptionally tight. The charge is transferred from the film to the capacitor in the amplifier's feedback loop, which determines the output voltage: $V = Q/C_f$.

The charge amplifier requires an op amp having a high input resistance and low bias current. A high input resistance avoids bleed-off of the charge on the feedback capacitor, and low bias current prevents the feedback capacitor from charging and discharging at excessive rates. The layout of the charge amplifier circuit is critical. The op-amp casing must be well grounded and the inputs should be guarded and connected to the same ground as the casing.

A layout with guarded inputs is shown in Figure 46. Also, to prevent leakage noise from being amplified by the op-amp, the input cable should be terminated using a well-insulated stand-off connector.

Even with the above precautions, it is likely that the output voltage will drift. To compensate for drift, a reset switch is generally designed into the circuit to manually reset the output to zero at intervals. One technique is to place a reed switch in series with a resistor, which is in parallel with the feedback capacitor C_f . Activating the reed switch closes the switch, discharging the voltage stored in the feedback capacitor.

Another method is to use a MOSFET device in which the maximum output voltage and off-gate voltage determine the minimum gate voltage of the FET. In practice, a supply voltage greater than the amplifier voltage is applied to the gate of the MOSFET, thereby lowering its drain/source resistance and creating a current path for discharge of the feedback capacitor.

The third alternative is to place a bleed resistor across the feedback. This resistor creates a time constant ($C_f R_b$), which is independent of the film capacitance and can be accurately controlled.

Figure 46. Layout for guarding inputs

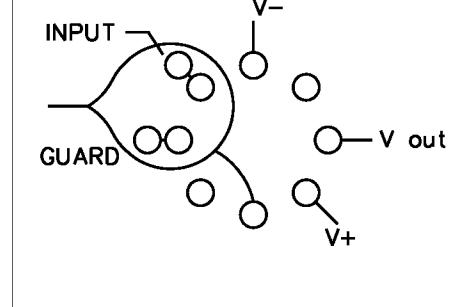
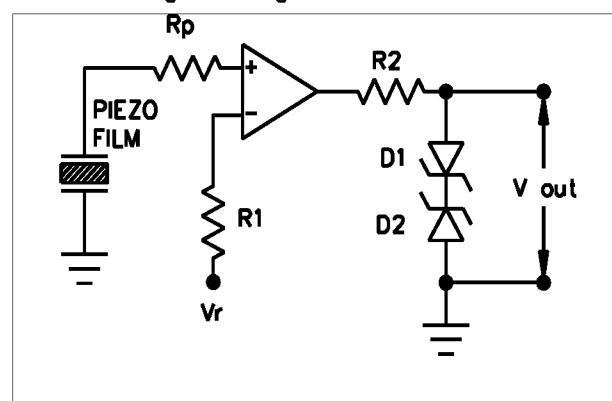


Figure 47. Signal level detector



The signal level detector of Figure 47 fits applications where large signal-to-noise ratios are desirable. This circuit is perfect for detecting an impact among low-level vibrations. For situations where signal to noise ratios are low and where impacts or pressures must be discerned from background vibration, the differential amplifier circuit of Figure 48 is appropriate. This circuit consists of two sensors driving a differential amplifier.

Figure 49. CMOS circuit for detecting a single impact

This configuration uses a common-mode rejection concept. The two switches are mechanically coupled to cancel unwanted vibrations that stimulate both. An input or pressure on one switch but not the other, will produce an output.

CMOS logic offers a low-cost way to interface with piezo film. As mentioned earlier, low-power circuits implemented with CMOS technology are ideally suited to piezo film switches. CMOS applications for piezo film are generally for low frequency operation. Other characteristics to consider include device input leakage current and input impedance, input bias resistance, and the effect of EMI. A CMOS circuit can be used, for example, in applications to sense a single impact or a single pressure.

The D-Flip Flop in Figure 49 indicates the presence of either the impact or pressure to set off an audible alarm.

The circuit in Figure 50, senses multiple impacts or pressures for counting applications.

Many different CMOS circuit configurations are possible to interface with piezo film. Common to all of them is an input bias resistor in parallel with the piezo film, and an input resistor in series with the film. The bias resistor handles leakage current and the series resistor limits current to protect against electrostatic discharge.

Cables

In applications where it is not possible to place the amplification circuit in close proximity to the piezo film transducer, considerable care must be exercised in selecting the connecting cable that carries the high-impedance signal.

Shielded coaxial cable, while used for noise reduction, can add problems associated with cable leakage and added capacitance. In most cases the cable's primary insulation should consist of highly resistant, non-polar plastics such as high-purity polyethylene or Teflon® (PTFE). It is equally important to make the cable as vibration-free as possible since cable movements generate noise that interferes with signal transmission.

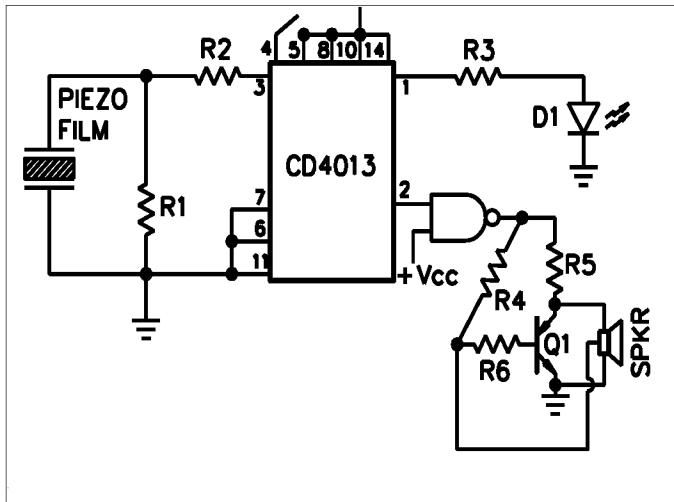
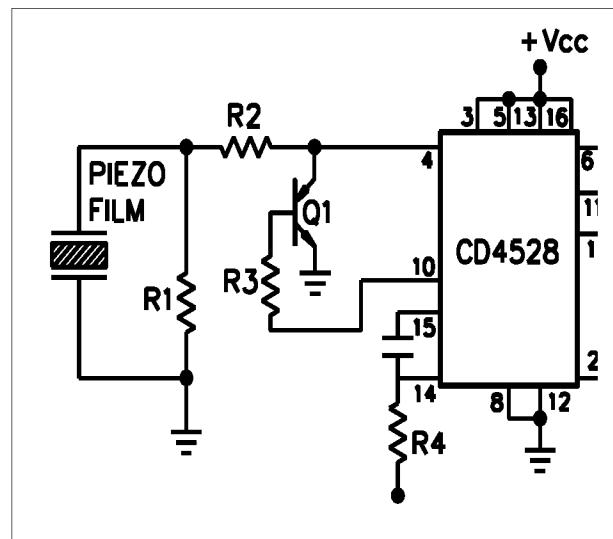


Figure 50. CMOS interface circuit for counting applications



MANUFACTURING

Rolls of piezo film are produced in a clean room environment. The process begins with the melt extrusion of PVDF resin pellets into sheet form, followed by a stretching step that reduces the sheet to about one-fifth its extruded thickness. Stretching at temperatures well below the melting point of the polymer causes chain packing of the molecules into parallel crystal planes, called "beta phase". To obtain high levels of piezoelectric activity, the beta phase polymer is then exposed to very high electric fields to align the crystallites relative to the poling field. Copolymers of PVDF are polarizable without stretching.

Evaporatively deposited metals are typically 500 to 1000 Å in thickness, and almost any metal can be deposited. Popular metals are nickel, aluminum, copper, gold and alloys. Electrode patterns are made by sputtering through masks or by chemical etching continuous metallizations using photoresists. Resolution to 25 µm line widths has been achieved. Screen printed electrodes of conductive silver ink are much thicker, about 5-10 µm, and can be applied in complex patterns to form multiple sensors on a single sheet. Foils are adhered with thin adhesive layers and capacitively coupled to the piezo film. Each electrode alternative has advantages and disadvantages.

Generally, sputtered metals are for very high resolution arrays, pyroelectric applications requiring a low thermal mass, or for inertness, as with invasive medical applications. Fully metallized sheets can be carefully cut with a razor blade without shorting across the film thickness. Screened inks are very robust and compliant, withstand very high strains (>10%), can operate at high voltages without breakdown, and are easy to pattern on a continuous basis. However, unmetallized borders are required for cutting elements out of a sheet of screen printed electrodes, since there is a high likelihood of shorting across the film thickness with the thick inks. Foils may mechanically restrict the piezo film from responding to externally applied stresses and strains in the plane of the film, but foils are useful in pure "thickness mode" operation.

After metallization, a wide variety of possible processing steps are followed to produce a packaged sensor. Generally, the piezo film is laminated in a protective carrier film, die cut to size, and packaged with lead wires or crimp connectors and, often, signal conditioning electronics. The wide range of packaged sensors, from a few square millimeters (including an ASIC chip) as a shipping damage sensor, to multiple square meter sensors for sports scoring targets suggests the versatility of this technology.

APPLICATIONS

The sensor applications described below represent a good cross-section of the products now using piezo film sensors.

Switches

The reliability of contact switches is reduced due to contaminants like moisture and dust which foul the contact points. Piezo film offers exceptional reliability as it is a monolithic structure, not susceptible to this and other conventional switch failure modes. One of the most challenging of all switch applications is found in pinball machines.

A pinball machine manufacturer uses a piezo film switch manufactured by MSI as a replacement for the momentary rollover type switch. The switch is constructed from a laminated piezo film on a spring steel beam, mounted as a cantilever to the end of a circuit board.

The "digital" piezo film switch features a simple MOSFET circuit that consumes no power during the normally-open state. In response to a direct contact force, the piezo film beam momentarily triggers the MOSFET. This provides a momentary "closure" for up to a 50 V maximum voltage. The output of this low profile contactless switch is well suited to logic-level switching. The unit does not exhibit the corrosion, pitting or bounce that are normally associated with contact switches.

The company has tested these switches in excess of 10 million cycles without failure. The switch solves the nagging problem of fouled contacts in pinball machines, a significant source for machine downtime and lost revenue. The simplicity of the design makes it effective in applications which include:

- Counter switches for assembly lines and shaft rotation
- Switches for automated processes
- Impact detection for machine dispensed products
- Panel switches
- Foot pedal switches
- Door closure switches

The cantilever beam that carries the piezo film can be modified to adjust switch sensitivity for high to low impact forces. Figure 51 shows the construction of the digital switch.

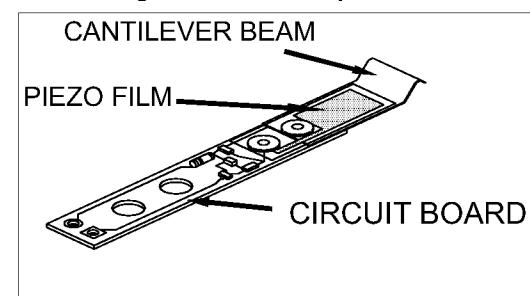


Figure 51. Switch for pinball machine

Beam Switch

Piezo film switches can be used to measure the amplitude, frequency and direction of an event and are useful in object detection and recognition, counting, wakeup switches and bidirectional encoding applications. The construction of the beam-type switch is shown in Figure 52.

Note that the piezo film element is laminated to a thicker substrate on one side, and has a much thinner laminate on the other. This moves the neutral axis of the structure out of the piezo film element, resulting in a fully tensile strain in the piezo film when deflected downward, and a fully compressive strain when deflected in the opposite direction. Were the neutral axis in the center of the piezo film element, as would be the case if the two laminae were of equal thickness, the top half of the piezo film would be oppositely strained from the bottom half under any deflection condition, and the resulting signals would therefore be canceled.

Beam switches are used in shaft rotation counters in natural gas meters and as gear tooth counters in electric utility metering. The beam switch does not require an external power source, so the gas meter is safe from spark hazard. Other examples of applications for the beam switch include a baseball target that detects ball impact, a basketball game where a hoop mounted piezo film sensor counts good baskets, switches inside of an interactive soft doll to detect a kiss to the cheek or a tickle (and the sensor is sewn into the

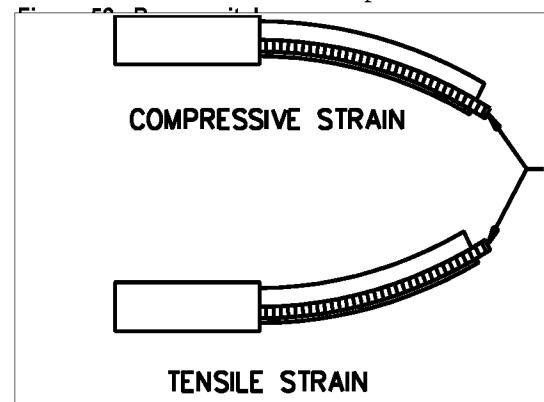


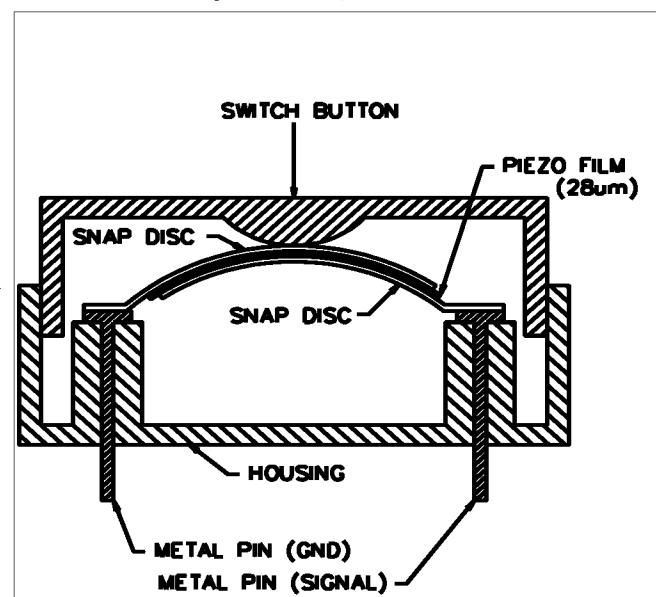
Figure 53. Snap-action switch

fabric of the doll), coin sensors for vending and slot machines and as digital potentiometer for high reliability.

Snap-Action Switches

Piezoelectric materials do not have a true dc response. Very slow events, 0.0001 Hz, for example, are not normally possible to detect with piezoelectric film. In switch applications where piezoelectric film in combination with a snap dome provides a high voltage pulse.

When the snap device actuates, the film is rapidly strained, typically generating a 10 volt pulse into a one megohm circuit as shown in Figure 53. This concept is especially well suited for wakeup switches, where an electronic device can be dormant for long periods without power consumption until the snap action device is actuated. The piezoelectric pulse turns on the electronics. Battery operated parking meters, where battery life is very critical, are an example of a piezo snap action switch application. A thermal snap action device also employs this principle.



Impact Sensors

Impact Printers

High speed impact printers require very accurate print head timing. Impact must occur the instant that a high speed revolving steel band, embossed with print characters, is properly positioned in front of the print hammer. Any advance or delay in energizing the print hammer will result in an offset print of the desired character.

Piezo film sensor strips, built into the printer platen, monitor the impact timing and force of the bank of print heads, and transmit the information to the controller. Automatic adjustment is made in the actuator timing to accommodate any minor change in print head timing. The very high speed of the embossed steel ribbon, about 300 inches per second, requires a very fast switch response. Alternative impact switches are quickly destroyed by the large impact forces of the print head. Piezo film switches have been in use in this application for more than five years without failure.

Sports Scoring

Piezo film sensors can be used to measure impact time, location (accuracy) and force. These parameters are desirable in several sports scoring applications. The energy of a 90 mph pitch has instantaneous power of about 50,000 watts! The great challenge in this application is target ruggedness without the introduction of severe bounceback into the design.

A second sports scoring application is electronic dartboards, where piezo film monitors the many impact zones in the game. Scoring is electronically recorded.

Musical Instruments

The popularity of electronics for musical instruments presents a special problem in drums and pianos. The very high dynamic range and frequency response requirements for drum triggers and piano keyboards are met by piezo film impact elements. Laminates of piezo film are incorporated in foot pedal switches for bass drums, and triggers for snares and tom-toms. Piezo film impact switches are force sensitive, faithfully duplicating the effort of the drummer or pianist. In electronic pianos, the piezo film switches respond with a dynamic range and time constant that is remarkably similar to a piano key stroke.

Figure 54. Permanent In-The-Road Traffic Sensor

The U.S. Government is actively studying "smart highways" as an alternative to major new highway construction. The idea is that existing highways can accommodate greater vehicle densities if electronically managed. In addition to conventional traffic monitoring for highway studies and enforcement, the Intelligent Vehicle/Highway System (IVHS) programs create the need for new classes of "smart highway" high speed sensors to count and classify vehicles, provide lane control, and to monitor weight and speed. IVHS also requires "smart car" sensors, and advanced vehicle surveillance, communications, and software.

Futuristic programs like the IVHS, and more contemporary projects like the Strategic Highway Research Program (SHRP), require traffic data collection to provide the necessary information required by the Federal Highway Administration on highway structures. Recent advancements in signal processing open the door to greatly improved real-time vehicle data analysis, provided that inexpensive reliable sensor technologies are developed.

Pneumatic road tubing has long been the workhorse of traffic data collection. Road tubes provide a pneumatic pulse to a piezoelectric membrane, which triggers nearby electronics when an axle is detected.

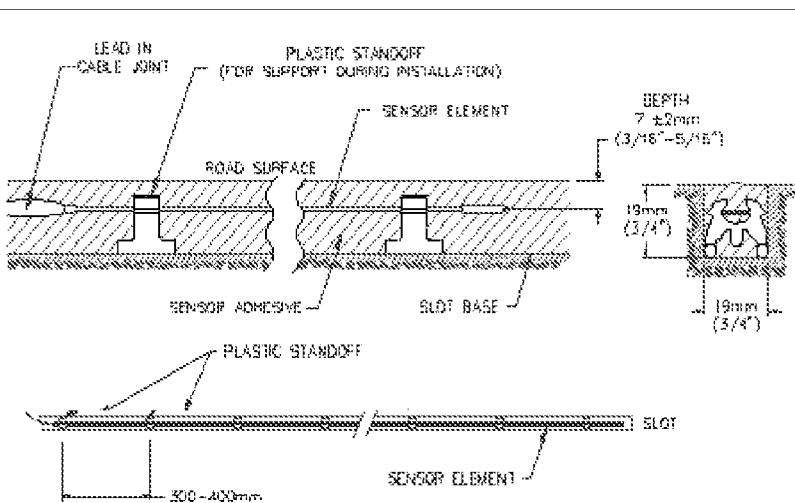
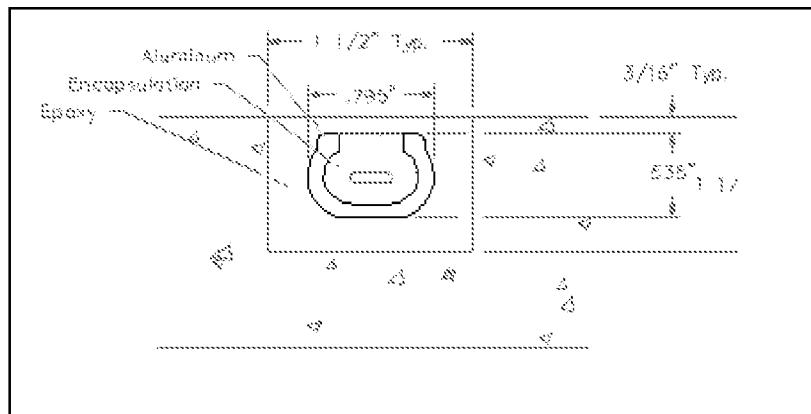


Figure 55. Permanent, In-The-Road Traffic Sensor

The evaluation of alternative sensor technologies has shown piezo cable provides the necessary sensitivity, linearity, noise immunity and environmental stability for high traffic interstate vehicle

classification and weight-in-motion systems. Piezo cable BL sensors are used for traffic data collection from Saskatchewan to Florida.

Piezo cable traffic sensor constructions are shown in Figures 54 and 55. There are two basic categories of traffic sensor ... permanent and temporary. Generally, permanent sensors are mounted in the road with the top of the sensor flush to the highway surface, while temporary units are adhesively applied to the road surface for shorter monitoring periods. Permanent sensors, used for toll booths and interstate highway data collection, are flush mounted to a road surface and must withstand the rigors of years of high density traffic, snow plows, salt, sand, water and dragging mufflers.

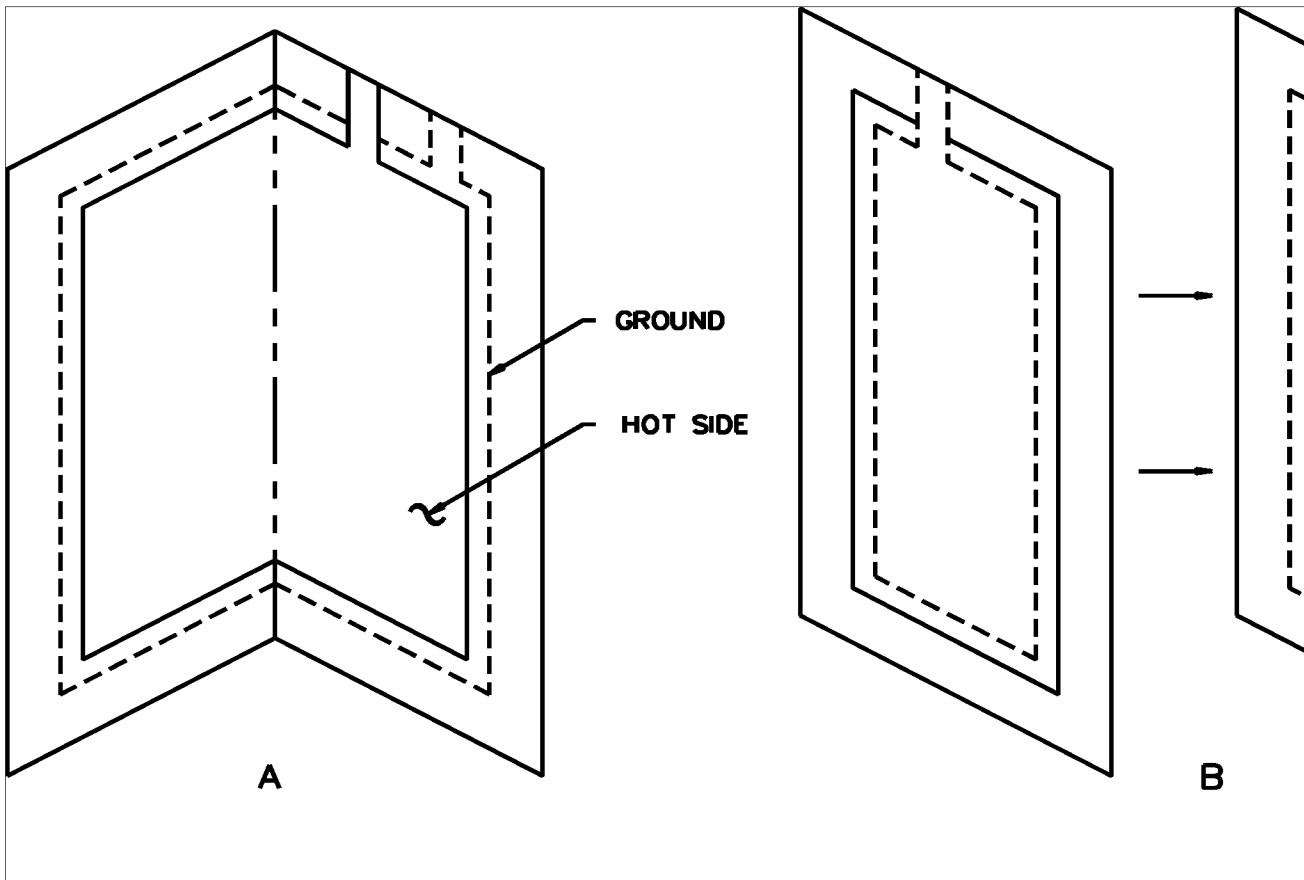
Traffic sensors can monitor vehicle speed, count axles, weigh vehicles, provide direction, and vehicle classification. Recently, these sensors have also proven valuable on airport taxiways. From the output, one can discern the ground speed of an aircraft (time lag between two sensors), its direction, weight (fueled), number of axles, and the span of the aircraft (determined from the speed and the known fixed distance between sensors). This information can be used to classify the aircraft and provides taxiway traffic control and safety information at airports.

VIBRATION SENSING

One of the first applications for piezo film was as an acoustic pickup for a violin. Later, piezo film was introduced for a line of acoustic guitars as a saddle-mounted bridge pickup, mounted in the bridge. The very high fidelity of the pickup led the way to a family of vibration sensing and accelerometer applications.

Music Pickups

Piezo film is used today in several guitar pickup designs; one is a thick film, compressive (under the saddle) design; another is a low cost accelerometer, while another is an after market pickup design that is taped to the instrument. Because of the low Q of the material, these transducers do not have the self-resonance of hard ceramic pickups. Shielding can be achieved by a foldover design as shown in Figure 57. The hot side is the slightly narrower electrode on the inside of the fold. The foldover technique provides a more sensitive pickup than alternative shielding methods because the shield is formed by piezoelectric material. Conventional shielding laminates can be easily fabricated by a multilayer laminate of piezo film, adhesive and shielding foil.



Machine Monitoring

The fidelity of a shielded piezo film sensor in musical instruments led to the development of vibration sensors for machines. In its simplest mode, piezo film vibration sensors behave essentially like dynamic strain gages. The film does not require an external power source, yet typically generates signals greater than strain gages **after** amplification. A typical piezo film sensor produces four orders of magnitude higher voltage signal than a foil-type strain gage, and two orders higher than semiconductor types. The frequency response of the piezo film strain gage is also superior.

The extreme sensitivity is due to the form of the piezo film material. The low thickness of the film results in a very small cross sectional area. Thus very small longitudinal forces create very large stresses within the material.

Piezo film sensors can be affixed to a vibrating surface and monitor the amplitude and frequency of the vibrating structure. The sensors can cover larger areas than normal strain gages so any direct comparisons should be performed in **uniform** strain fields for meaningful results. Obviously, point-type transducers may be used where required, although the low capacitance of the small sensor area will require additional consideration. Operation down to fractions of Hz can be achieved by either conventional charge amplifiers or, since signal levels are relatively high, simple high impedance FET buffer circuits.

Bearing Wear Sensors

A shielded piezo film sensor has been used to monitor bearings for wear and evidence of spall. The sensors are permanently affixed to the outer surface of the bearing race with epoxy. The low mass and thin profile allow its use as a built-in nondestructive testing sensor, rather than the time consuming use of accelerometers for periodic fault-condition checks.

Fan Flow Sensor

A laminated beam type sensor is used in ducted airflow as a centrifugal fan failure sensor. The presence of air flow is detected by the vibrations in the sensor caused by the turbulence of air flow at about 100 Hz. The absence of this signal is used for trigger electronics. The sensor and switching electronics are based around a TL084 quad bi-FET op amp, with typical input signals of 80 mV. Sensor reliability is the key feature. Since the sensor is subjected to virtually no operating stresses, it has an indefinite working life.

Vending Sensors

Shielded dynamic strain gages of piezo film are affixed to the underside of a vending product delivery tray to verify that product was properly vended. The absence of the impact induced vibration triggers an "Out of Order" warning. In a second application, slot machine coin counting is provided by a piezo film element. The sensors confirm delivery of coins won, discouraging gamblers from falsely claiming equipment defects. A ticket dispensing machine counts tickets delivered with a piezo beam design. Coin sensors also trigger or wake-up vending machines and coin changer electronics to verify coin authenticity.

ACCELEROMETERS

A logical outgrowth of the many vibration sensor applications of MSI's piezoelectric technology are accelerometers. These accelerometer designs are based on more traditional piezoelectric ceramic, as well as piezoelectric polymer materials. The choice of base materials allows the product to be tailored for specific applications. Table 6 lists the key specifications for the MSI Accelerometer product family.

Like more conventional sensors, these accelerometers are configured as either compression-design type or beam-design type. Compression-design accelerometers typically have higher resonant frequencies providing wide useful frequency ranges. Consequently, they

also tend to have lower sensitivities. An internal view of MSI's ACH-01-XX compression-design accelerometer is shown in Figure 59.

Beam-design accelerometers, on the other hand, tend to have lower resonant frequencies and useful frequency ranges but higher sensitivities. Beam-design accelerometers also have another very interesting feature: They can be oriented to sense acceleration in multiple-axes with one monolithic sensing element using MSI's patented "Origami" beam technology ("Origami" is the Japanese

Figure 59. ACH-01-XX internal view

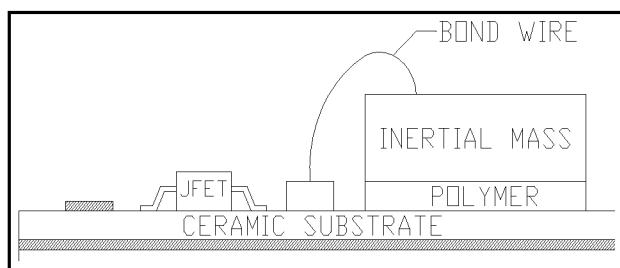
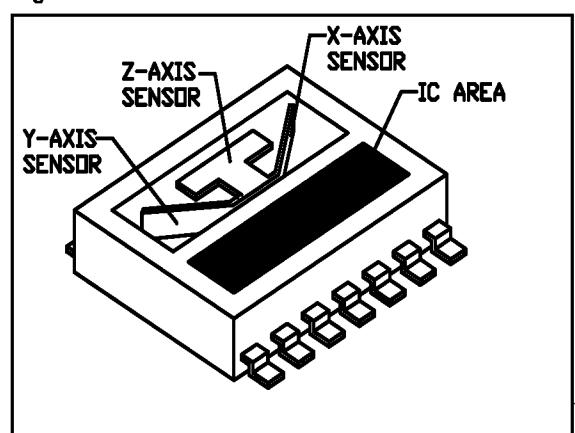


Figure 60. ACH-04-08-05 internal view



word for the art of paper folding). An internal view of the ACH-04-08-05 three axis beam-design accelerometer, with its origami sensing element, is shown in Figure 60.

To reduce system costs as well as simplify use, all of MSI's accelerometers include some type of integral electronic sensor interface. The ACH-01 family of accelerometers and the ACH-04-08-05 multiple-axis accelerometer both use simple JFET impedance buffers. With these sensors, JFET biasing and signal processing is implemented external to the device.

The ACH-01 family of products is typically used in applications which require broad frequency capability, high sensitivity, low noise, and low cost. Such applications include: speaker feedback and control systems, automotive anti-theft systems, acoustic pick-ups, machine-health and pump and centrifuge monitoring systems, and medical body motion monitoring.

The ACH-04-08 product family, because of the products wide capabilities, is used in a very broad range of applications such as computer hard-disk-drive shock sensing, speaker feedback and control systems, appliance fault monitoring, virtual reality systems, automotive systems, medical body motion monitoring, shipment damage and material-handling monitoring systems, GPS systems, vibration switches and earthquake shut-off switches. OEM applications that require acceleration or vibration measurements in more than one axis are perfect for the ACH-04-08.

MSI is constantly developing and upgrading its accelerometer product line. Please contact MSI for further details on these products or on customizing one of our other products.

Table 6. Accelerometer Family

Production Qualified Accelerometers			
		ACH-01-XX	ACH-04-08-05
Key Features		-Wide Frequency Range -Wide Dynamic Range -High Sensitivity -Low Noise -Very High Resonance -JFET Buffer IC	-Low Frequency Operation -3 Simultaneous Analog Outputs
Sensitive Axes	X-Axis	---	X
	Y-Axis	---	X
	Z-Axis	X	X
Sensitivity		9mV/g	1.8 mV/g
Frequency Range (± 3 dB)		2.0 Hz-20 KHz	0.3 Hz to 5 KHz
Dynamic Range		± 150 g	± 250 g
Resolution (@ 100 Hz)		6.25 μ V/ \sqrt{Hz} / GNDT	6.25 μ V/ \sqrt{Hz} / GNDT
Resonant Frequency		>35 KHz	9.2 KHz
Resonant Q (Hz/Hz)		30	10
Transverse Sensitivity		2%	10%
Linearity		0.1%	0.1%

Operating Temperature	-40 °C to +85 °C	-40 °C to +85 °C
Storage Temperature	-40 °C to +85 °C	-40 °C to +105 °C
Maximum Shock	1000 g	1000 g
Supply Voltage	3 V to 40 V	3 V to 40 V
Supply Current	150 mA (ACH-01-03)	200 mA (ACH-01-01)
Weight	8 grams (ACH-01-03)	0.35 grams
Size (mm)	13 x 19 x 6	11 x 10 x 1.8
Mounting Method	Adhesive	Hand Solder to PCB

Table 7. Accelerometer Applications

		ACCELEROMETER PRODUCTS	
Industry	Application	ACH-01-XX	ACH-04-08-05
<i>Aerospace & Defense Electronics</i>	Anti-Tamper Sensors	X	X
	Surveillance	X	
	Modal Analysis	X	X
<i>Automotive</i>	Vehicle Motion Sensing	X	X
	Antitheft	X	X
<i>Computers & Peripherals</i>	Disc Drive Shock Sensor		X
	Computer Mouse Sensor		X
	Signature Verification Writing Analysis Pen		X
	Virtual Reality Sensor		X
<i>Household Appliances</i>	Out-of-Balance Sensor	X	X
	Vibration Switch	X	X
<i>Consumer Electronics</i>	Speaker Feedback	X	
	Acoustic Pick-ups	X	
	Security	X	X
<i>Industrial</i>	Machine Health Monitor	X	X
	Bearing Monitor	X	X
	Impact Detection	X	X
	GPS Wake-up Switch		X
	Out of Balance Indicator	X	X
	Vending Verification Sensor	X	X
	Vibration Switch	X	X
<i>Instruments & Measuring Equipment</i>	Active Vibration Damping	X	X
	Vibration Switches	X	X
	Predictive Maintenance	X	X
<i>Medical</i>	Motion Sensor	X	X
<i>Power & Utilities</i>	Earthquake Shut-Off		X
	Machine Monitoring	X	X
<i>Telecommunications</i>	GPS Systems		X
	Vibration Switches	X	X
<i>Transport & Material Handling</i>	Shipment Monitoring	X	X
	Railroad Systems	X	X

ULTRASOUND APPLICATIONS

The wide frequency response and physical attributes of its polymeric construction makes piezo film a material of choice in certain medical probes and in nondestructive testing applications. Additionally, the film sensors are found in applications in ultrasonic based sensing devices, like air-ranging ultrasound for distance measurement, in fluid level sensors, and in-flow measurement instruments using doppler shift of sound velocity perturbations which are proportional to fluid flow.

Medical Imaging Ultrasound

Piezoelectric ceramic materials are used in medical imaging transducers because of their high sensitivity and broad bandwidth. The d_{33} constant, strain developed for an applied voltage, is about an order of magnitude higher for piezo ceramics than for piezo polymer. A disadvantage of piezo ceramic is its high acoustic impedance, about 30 MRayls ($1 \text{ MRayl} = 10^6 \text{ kg/m}^2\text{s}$) in contrast to about 1.5 MRayls for body tissue. This impedance mismatch can be compensated by quarter wavelength matching layers, but these can degrade the ultrasonic pulse due to adhesive layers and construction methods. The acoustic impedance of piezo film is about 4 MRayls, a much better match. Additionally, in higher frequency applications requiring very thin piezo elements, ceramics are too fragile, and cannot be shaped to desired geometries.

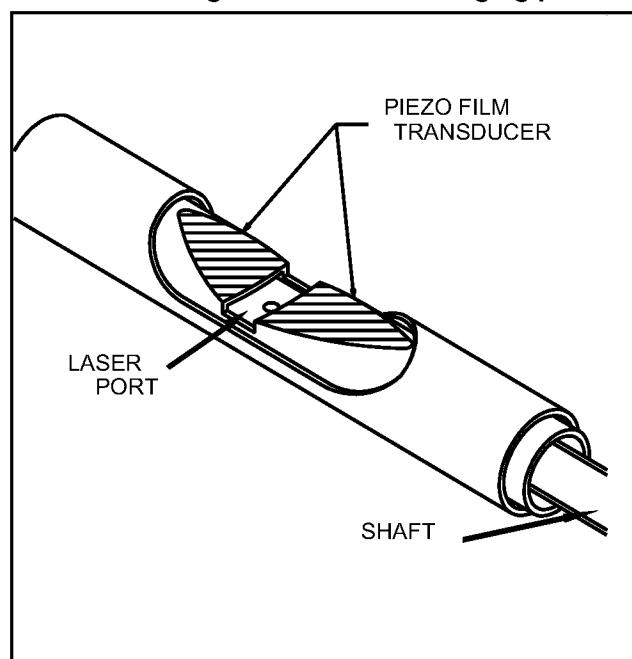
Invasive imaging requires lower powered devices than external probes. Resolution of the image is considerably improved at the higher frequencies of invasive catheters. A medical imaging company has developed an invasive imaging probe with piezo film for a therapeutic laser prostate catheter (Figure 61). The piezo film sensor is about 30 microns thick, and is located near the catheter tip. The unit operates at frequencies of 7 MHz and higher.

Steered *in-vivo* phased-array images using piezo polymer film have been produced for the first time by researchers at Duke University. A 32 element array of 11 mm x .56 mm elements was fabricated and tested with a well matched circuit designed to optimize the transducer. The result was 28 dB lower sensitivity than PZT transducers at 2.5 MHz operating frequency. However, the piezo film array had improved axial resolution, better angular response (6 dB pulse-echo response at 30 degrees), and a low interelement cross-coupling of -35 dB. It is exceptionally difficult to diamond blade saw

PZT ceramic into these small elements; while, for piezo film, complex patterns are readily etched into the surface gold electrode. PZT must be diced due to the severe interelement coupling problem. Duke University researchers plan to improve the polymer probe by expanding the number of array elements to 128.

Very high resolution arrays have been traditionally formed by etching an electrode pattern on the surface of a piezo film. Newer techniques include deposition of the copolymer directly onto silicon wafers. The wafers are etched to minimize interelement coupling, then the copolymer is applied by spin-

Figure 61. Invasive imaging probe



coating, followed by poling. Then a top ground electrode is applied and inter-connections made. This advance results in a very high resolution imaging. Capacitively coupling copolymer film to a dense array of conductive traces on a PCB has achieved remarkable performance as a Tx/Rx array.

NonDestructive Testing (NDT)

Advanced composite materials are very desirable as structural members. Light weight, high strength, corrosion resistance, and non-magnetic are among the advantages for these materials. The need for very routine nondestructive testing of such structures to prevent catastrophic failure due to delamination, is one of technology's greatest concerns. Flexible sheets of piezoelectric polymer transducer arrays, acoustically well matched to the composites, are desirable for use for non-destructive testing. One example is as an NDT array for testing rocket motor housings prior to launch. These arrays can be applied to the surfaces of composite fuel housings, and each element sequentially activated to provide a pulse-echo response. An array element size of about 0.5 to 1 square inch is sufficient for this application, as well as most large area NDT. Center frequencies of 3-10 MHz and -6 dB fractional bandwidths exceeding 100% are typical with such transducer arrays.

Systems and Instrumentation, Ltd. personnel use piezo film for NDT of aerospace engine parts. NDT transducers capable of detecting flaws down to 1/64th inch are now required. Further, the frequency response range of these new materials are broader than the bandwidth of conventional transducers. S&I, Ltd. find that a single broadband transducer covers the bandwidth of interest. Their transducers are also used in near-surface NDT applications, where high resolution and short pulse duration are required. Defects of 0.8 mm in size, lying within 1 mm of the surface, have been detected with the S&I probes.

Critical points or inaccessible test areas within a composite structure, like support strut mounts, where delamination or other damage is especially likely, can have custom fabricated NDT arrays permanently affixed for in-service testing and monitoring. It is possible to achieve uniformity of ± 1 dB between the elements of a multi-element transducer array. Special shaped transducers, providing special focal characteristics, have also been built with these polymer transducers.

Acoustic Emission

Acoustic emission of materials including fiber-reinforced composites, aluminum, steel and glass can be performed with contact microphones of piezo film, or, as with NDT, by large area arrays. These arrays can continuously monitor structures for 0.1 - 1.0 MHz acoustic emission, the precursor to structural failure. Piezo film, being broad band, responds well at these frequencies. This capability is especially necessary for critical application like tank rail cars carrying toxic products, underground fuel storage tanks, nuclear plants, etc.

Fluid Level Sensor

There are a variety of fluid level sensing transducer technologies available to the designer. A float arm, attached to a sliding potentiometric device is still widely used in automobiles. Ultrasonic pulse-echo devices that measure the distance from a fixed transducer to the fluid surface from above through air, or from below through the fluid, are popular. Newer capacitance types, where the fluid becomes the dielectric, are also used. Each of these technologies represent tradeoffs in system cost, performance and reliability. A new ultrasound level sensor, in development by MSI, holds promise as a digital, solid state ultrasonic level sensor.

The novel construction is a level sensor with ultrasonic through-transmission with multiple transmitters and a single, common receiver. The sensor is fabricated by attaching an unmetallized strip of piezo film to a printed circuit board containing electrode patterns, conductors, and interconnections to circuitry on the opposite side of the board. The electrode patterns are capacitively coupled to the piezo film layer,

becoming the multiple transmitter elements. A second conductor bar, parallel to the patterned elements becomes the common receiver.

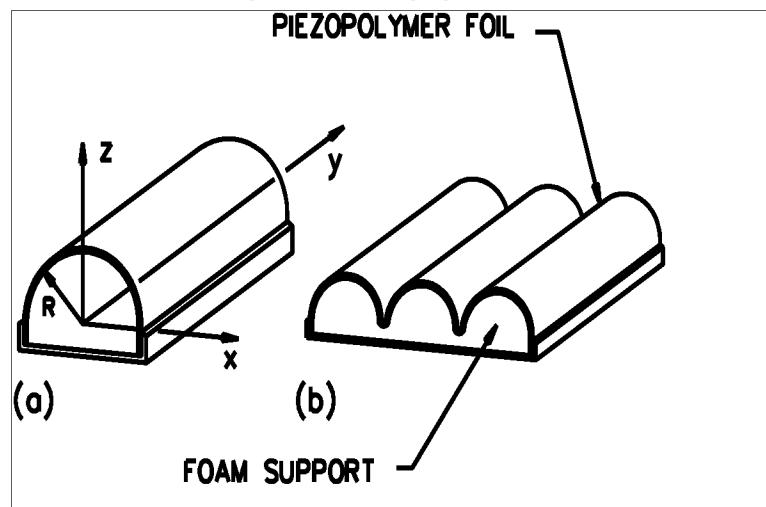
The presence of fluid couples the transmit signal to the receiver to a much greater extent (60dB) than when the ultrasound energy is coupled by the air above the fluid. The excitation signal for the transmitter is a 1.1 MHz sine wave tone burst with an amplitude of 20 volts peak to peak. The required circuitry consists of a high frequency oscillator and clock, an array of analog switches, a single receiver amplifier with input gate, and a threshold detector. These electronics can be reduced to the chip level, and are incorporated on the backside of the circuit board.

Resolution of the level sensor is determined by the resolution of the patterned transmit electrodes on the circuit board. Parallel elements of 2 mm width and 0.5 mm spacing between elements is a representative capability. The ground electrode for the transmitters is a fully gold metallized surface on the fluid side of the piezo film transmitter array. The receiver is formed by the same piece of piezo film, capacitively coupled to the signal electrode which is a separate conductor trace on the printed circuit board (PCB). Again, the ground is the backside electrode on the film.

The new level sensor has several unique advantages. The spacing between transmitter elements need not be uniform. For tanks that do not have a uniform volume throughout the tank height, a simple PCB layout can linearize the nonlinear tank volume by setting the transmitter element spacing accordingly.

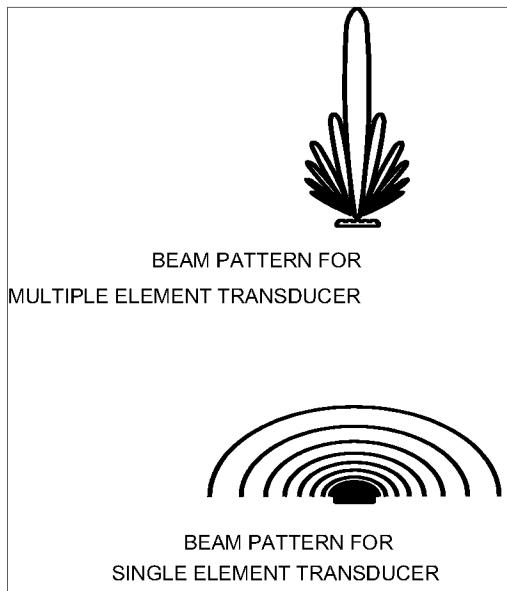
The output of the device is digital—no expensive A/D conversion is required. The level sensor is small in width, less than 1 inch, so it can be inserted into a small diameter tube. The tube confines the motion of the fluid, reducing large swings in fluid height readings caused by motion, as with an automobile fuel tank during cornering. Reliability is greatly improved. The level sensor is self diagnostic to the extent that the transmitter/receiver pair must be operational to deliver a meaningful signal. The absence of the signal indicates a fault condition. For a detailed discussion on Ultrasonic Ink Level Sensing, see Appendix C.

Figure 62. Air ranging ultrasound transducers



Air Ranging Ultrasound

Ultrasonic devices used in pulse-echo modes are used in robotics, vehicle safety and control system, object recognition systems and other remote distance measurement devices. The sensors provide high resolution in the targeted direction, and can be used to measure the elapsed time from transmit to receive to determine the distance to an object. Unlike piezo ceramic and electrostatic devices, piezo film can deliver a very short pulse (due to its low Q), allowing the same device to be used as both transmitter and receiver, even in the near field of the transducer.



of transducer sensitivity are 0.1-1 mV/Pa in the receive mode (noise was $< 1\mu\text{V}$) and 15-75 mPa/Vcm² in the transmit mode for 1 m of distance. The minimum distances measured in pulse-echo mode was 30 mm. Distances to 15 meters have been measured with a main beam width of less than 10 degrees, and maximum side lobe amplitudes that are 12 dB down at 60 KHz. Examples of directivity patterns for single and multiple element transducers are shown in Figure 63. Multiple elements can also be used for scanning of objects without physically moving the transducer. Each element within the transducer can be activated sequentially, as with ultrasonic arrays.

Multiple piezo film elements can be easily fabricated, as shown in Figure 62. The geometries of these cylindrically shaped elements (length, radius of curvature, number of elements) can be designed to control the directivity pattern and acoustic properties. Transducers with operating frequencies from 40-200 KHz have been made. Average values

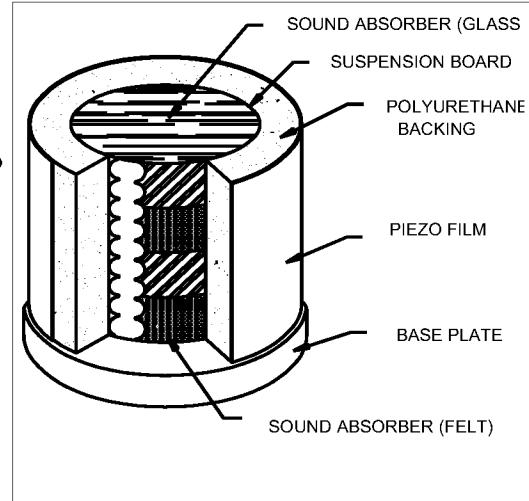
Figure 64. Audio speaker

AUDIO

Speakers

One of the earliest applications for piezo film was in stereo tweeters (Figure 64) and headset speakers developed by Pioneer Electronics. There is strong renewed interest in these applications as a result of the improvements in the reliability of the electrodes and lead attachment and packaging techniques. Gallo Acoustics has developed a high fidelity omnidirectional tweeter using a cylinder of 52 μm thick piezo film. The tweeter rolls off at frequencies below 2 KHz, and features:

- 330 degrees of horizontal dispersion at high frequencies, which is as much as ten times the dispersion of conventional tweeters,
- very wide dynamic range,
- linear frequency response,
- very fast impulse response, faithfully reproducing the highest frequencies.



Novelty audio speakers have also been developed. These devices make use of the thin, light weight, conformal nature of the piezo film. Examples include speakers for inflatables (like balloons and air inflatable toys), speakers in apparel (including headgear) and paper thin speakers for magazine advertising, greeting cards and posters.

Micropohones

A diaphragm of piezo film, affixed in a retaining ring or mounted over a hole in a plate, makes an excellent microphone. Vacuum formed domes on a support can be introduced into the design to take the membrane slightly out of its neutral axis with a foam backing, a small post, bar or structure to give the film membrane a slight radius of curvature. A self-supporting, cylindrically curved film also achieves the mechanical bias. A typical radius of curvature for piezo film microphones which optimizes sensitivity and electroacoustic efficiency is $R_o = 25$ mm, similar to that of an electrostatic microphone construction.

Sennheiser reports a frequency response for a typical foam backed piezo film microphone of 25 mm diameter, having $R_o = 25$ mm. The free field sensitivity of the device measured at 1 KHz, for sound pressure incident on the membrane perpendicularly, was -58 dB re 1 V/Pa. Harmonic distortion approaches 1% only at sound pressure levels exceeding 122 dB, and are not significantly higher for the range of higher frequencies.

Microphones built with piezo film are low cost, but more importantly, are inherently immune to moisture, unlike electrostatic types.

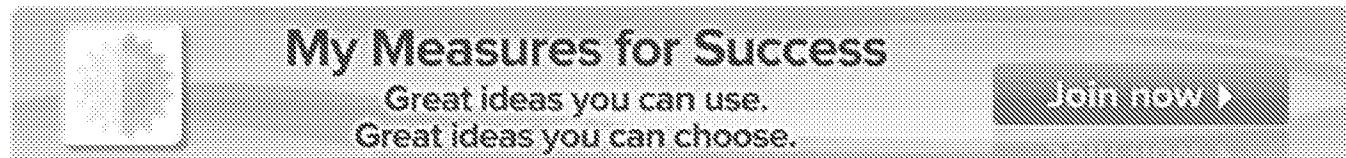
Electrostatics dominate the market due to the low cost that has been achieved through very high volume manufacturing. Nonetheless, piezo film microphones are finding application in designs where environmental stability is critical. Waterproof microphones are being supplied for divers, withstanding total immersion in salt water without damage.

Appendix A – Applications of Piezo Film

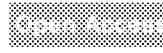
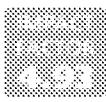
APPLICATIONS OF PIEZO FILM

<u>COMPUTER INPUT/OUTPUT</u>		
<i>Keypad arrays</i>		<i>Physical Security & Energy Management</i>
Digitizer		Glass Break Detectors
Air Mouse		Floor/Mat Sensor
Joystick		Penetration Detection
Pen (Signature verification; Handwriting		Contact Microphone
Recognition)		Piezo Cable Perimeter Protection
<i>Printers</i>		Pyrometer/Flame Sensor
Impact Flight Time	Ink	<i>Flow/Level</i>
Drop Generation and Detection		Vortex
Toner and Ink Jet Level		Fluidic Oscillator
Toner Activation		Air Flow
<i>Business Equipment</i>		Doppler Ultrasound
Antitamper for ATM Machine		Solid State Fluid Level
Coin Counters		Laminar/Turbulent Boundary Layer
<i>Copiers</i>		Fan Failure
Switches		<u>INSTRUMENTATION</u>
Paper Path Switches		<i>Machine Health Monitor</i>
Toner Level and Activation		Accelerometers
<i>Disc Drives</i>		Contact Microphones
Shock Sensing Accelerometers		Hi-Strain Dynamic Strain Gages
<u>INDUSTRIAL</u>		<i>Weather Sensors</i>
<i>Switches</i>		Rain Intensity
Solid State Momentary		Hail Detection
Snap Action		Wind Velocity
Cantilever Beam		<i>Active Vibration Damping</i>
Keypad		Strain Gages Sensor Arrays
Vandal-Proof		Actuator Arrays
Intrinsically Safe		<i>Non Destructive Engineering</i>
CMOS Wake-up		Flexible Contact NDT Probes
Low-Deflection		NDT Arrays
Singing Switch (a.c. switch)		Acoustic Emission Sensors
Coin Counter		<i>Air Ranging Ultrasound</i>
Acoustic Switch		Safety
Shaft Rotation Counter		Distance
<i>Robotics</i>		<i>Adaptive Optics</i>
Tactile Sensor		Fiber Optic Shutters/Modulators
Micropositioner		Deformable Mirrors
Safety Mats & Switches		Laser Scanners
Bumper Impact		<i>Oil Exploration</i>
		Hydrophones
		Seismic Geophones

<u>MEDICAL</u>	<u>CONSUMER</u>
<i>Diagnostics</i>	<i>Musical Instruments</i>
Apnea Monitor	Piano Keys
Ambulatory/Gait Monitors	Pick-up
Blood Pressure Cuff	Drum Trigger
Pulse Counter	<i>Sports Equipment</i>
Stethoscope	Target Location
Sleep Disorder Monitors	Reaction Time
Respiratory Air Flow	Foul Line
Isokinetics	Force (Karate, Impact)
Patient Bed Monitor	Sweet Spot
<i>Ultrasound</i>	<i>Toys/Games</i>
Near Field Imaging	Switches
Prostate	Proximity (Air Ranging Ultrasound, Pyro)
Transdermal	Novelty Speakers (Microphones)
Transluminal	Target Scoring
Coronary Arterial	<i>Audio</i>
Breast	Tweeter
Lithotripter	Balloon Speakers
Hydrophone Calibration Probes	Novelty Speakers (Visor, Poster)
<i>Handicapped Aides</i>	Microphone
Switches	Speaker distortion Feedback
Braille Reader	<i>Accelerometer</i>
Hearing Aid	Washer Imbalance
Speech Intensification	Vacuum Soil Sensing
<i>Implantables</i>	Dishwasher Spray Arm
Pacemaker Activity Monitor	Level Sensing Switches
Implantable Switch	
Vascular Graft Monitor	
Micropower Source	
<i>Instrumentation</i>	
Intravenous Drop Counter	<u>MILITARY/GOVERNMENT</u>
IV Air Bubble Detection	<i>Hydrophones</i>
Laser Switch/Modulator	Towed Cable Array
	Hull Mounted Arrays
	Sonobuoys
	Active Noise Suppression
<u>AUTOMOTIVE</u>	<i>Ballistics</i>
Accelerometers	Safety and Arming Fuses
Occupancy Seat Sensor	Shock Wave Gages
<i>Switches</i>	Seismic Accelerometers
Passenger Compartment Switches	<i>Physical Security</i>
Horn Switch	Perimeter Security Cable (Buried or
Control Panel	Fence)
<i>Fuel Level, Tire Rotation, Security</i>	Seismic/Geophones
Keyless Entry	Covert Microphones
Motion (Theft) Sensor	<i>Traffic Sensors</i>
	Vehicle Classification
	Weight-In-Motion
	Speed, Red Light Enforcement
	Lane Designation
	Toll Booth



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Lack of agreement between bioimpedance and continuous thermodilution measurement of cardiac output in intensive care unit patients

Ben N Barry¹, Abhiram Mallick¹, Andrew R Bodenham² and Michael Vucevic¹

¹ Academic Unit of Anaesthesia, The General Infirmary at Leeds, Great George Street, Leeds LS1 3EX, UK

² Intensive Care Unit, The General Infirmary at Leeds, Great George Street, Leeds LS1 3EX, UK

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Keywords: measurement techniques, impedance cardiography, thermodilution, monitoring, cardiac output

Abstract

Background

Bolus thermodilution is the standard bedside method of cardiac output measurement in the intensive care unit (ICU). The Baxter Vigilance monitor uses a modified thermodilution pulmonary artery catheter with a thermal filament to give a continuous read-out of cardiac output. This has been shown to correlate very well with both the 'gold standard' dye dilution method and the bolus thermodilution method. Bioimpedance cardiography using the Bomed NCCOM 3 offers a noninvasive means of continuous cardiac output measurement and has been shown to correlate with the bolus thermodilution method. We investigated the agreement between the continuous bioimpedance and continuous thermodilution methods, enabling acquisition of a large number of simultaneous measurements.

Results

A total of 2390 paired data points from seven patients were collected. There was no correlation ($r^2 = 0.01$) between the methods. The precision (1.16 l/min/m^2) of agreement between the Vigilance and the Bomed, assessed by the Bland-Altman method, was very poor although the bias (-0.16 l/min/m^2) appeared fair.

Conclusions

The Bomed NCCOM 3 bioimpedance monitor shows poor agreement with the Baxter Vigilance continuous thermodilution monitor in a group of general ICU patients and cannot be recommended for cardiac output monitoring in this situation.

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Introduction

The fluid bolus thermodilution method of cardiac output measurement, using a pulmonary artery catheter (PAC), has gained wide acceptance over the past 25 years. The advantages and disadvantages of the use of this method of monitoring critically ill patients are well established [1].

A recent development has been the introduction of 'continuous' cardiac output monitoring using a modified PAC (Continuous Cardiac Output/SvO₂ Catheter model 746H8F, Baxter Healthcare Corporation, Round Lake, Illinois, USA). This catheter has a thermal filament that produces pulses of heat at the level of the right ventricle, and a thermistor at the tip in the pulmonary artery senses temperature change. A dedicated computer (Vigilance, Baxter Healthcare Corporation) is required, which updates calculated cardiac output every 30–60 s. This system has been previously investigated [2] and has shown a very strong correlation with both the 'gold standard' dye dilution technique ($r^2 = 0.91$) and fluid bolus thermodilution ($r^2 = 0.97$). It has also been evaluated specifically for use in critically ill patients [3] and in a bench model of pulmonary artery blood flow [4].

Bioimpedance cardiography has been developed over the past 30 years as a noninvasive technique to measure cardiac output. Monitors such as the Bomed Noninvasive Computerized Cardiac Output Monitor (NCCOM 3, Bomed Medical Manufacturing Ltd, Cheshire, UK) are commercially available to measure cardiac output. However, in the United Kingdom and elsewhere they have not achieved widespread usage. The NCCOM 3 uses eight spot electrodes, placed at the root of the neck and chest wall. A constant sinusoidal alternating current (2.5 mA rms, 70 kHz) is passed through the subject's chest and the impedance measured. By measuring the maximum rate of change of thoracic impedance during systole, timed from the electrocardiogram (ECG), the stroke volume is calculated. The Sramek-Bernstein formula is used, which calculates stroke volume as the volume of electrically participating intrathoracic tissue \times ventricular ejection time \times index of contractility, which is the ratio of the peak rate of change in thoracic bioimpedance and the thoracic fluid index (or total thoracic impedance). Cardiac output is then calculated from the product of heart rate and stroke volume, averaged over 16 cardiac cycles.

We have investigated the correlation between these two methods of continuous cardiac output measurement to determine their suitability for use in critically ill patients in the intensive care unit (ICU).

Methods

We compared the Bomed NCCOM 3 with the Baxter Vigilance in a mixed group of seven ICU patients. All patients required pulmonary artery catheterization on clinical grounds. In two patients, an existing PAC was exchanged for a continuous cardiac output PAC, using the same introducer sheath. An explanation of the use of noninvasive bioimpedance monitoring as part of a research study was given to the patients' relatives and assent was obtained. The primary pathologies of the patients were acute pancreatitis (one), emergency repair of an abdominal aortic aneurysm (two), appendix abscess (one), probable pulmonary embolism (one), cholangitis following cholecystectomy (one) and respiratory failure (one). The study was purely observational, and required no alterations in therapy.

For bioimpedance cardiography, eight standard ECG gel electrodes and, if necessary, two additional electrodes for ECG monitoring were applied, according to directions printed on the NCCOM 3 monitor. Timed data points were saved on a personal laptop computer connected to the NCCOM 3 using the CDDP version SI 4.05 software (CDIc, Irvine, California, USA).

For thermodilution measurements, a continuous cardiac output PAC connected to the Vigilance monitor was inserted via the internal jugular or subclavian vein. Timed data was stored in the patient monitoring system (Hewlett Packard Ltd, Boise, Idaho, USA), using the 'Vue-link' software to connect the two devices. A print-out of cardiac output data at 1-min intervals was obtained at the end of the study period.

Any discrepancy between the clocks on the two monitors was accounted for by noting the times displayed at the start of measurement and allowing for this when pairing the data. In this way we ensured that the paired data points were accurately synchronized.

Body surface area was calculated by each device in order to obtain 'indexed' measurements, by entering the patients' height and weight, estimated if necessary. We verified that both devices produced the same body surface area, so eliminating this source of bias error.

Each patient was monitored for a period of approximately 6 h, acquiring simultaneous paired cardiac index data points at 1-min intervals. The data points were analysed using SPSS for Windows release 6.1 (SPSS Inc, Chicago, Illinois, USA) and r^2 was calculated using regression analysis. A plot of the difference between measurements against the mean of the measurements was then constructed according to the technique for assessing agreement between two methods of clinical measurement described by Bland and Altman [5].

Results

Seven patients were studied; the mean (\pm SD) age was 63 ± 16 years, mean weight 86 ± 31 kg and mean body surface area 2.0 ± 0.34 m 2 . A total of 2390 simultaneous paired cardiac index data points were analysed, with approximately equal numbers of data points from each patient. The patients were all mechanically ventilated; other ongoing supportive care included vasopressor and inotropic support and renal replacement therapy (continuous haemofiltration or intermittent haemodialysis) as required by the individual patient. Positive end-expiratory pressure (PEEP) was used as clinically indicated, up to 10 cm H $_2$ O. During the study none of the patients suffered new dysrhythmias requiring treatment or interfering with cardiac output measurement.

The range of cardiac index measurements was 1.40–7.20 l/min/m 2 (mean 3.50 ± 0.95 l/min/m 2), by the Bomed, and 1.60–5.60 l/min/m 2 (mean 3.65 ± 0.77 l/min/m 2) by the Vigilance monitor. There was essentially no relationship between the two methods ($r^2 = 0.01$; Fig 1). The correlation coefficients for individual patients were -0.25, -0.21, 0.41, 0.25, -0.39, -0.16 and 0.06, respectively.

A Bland–Altman plot (Fig 2) showed a poor degree of agreement between the methods. Although the degree of bias was acceptable, the precision was very poor. The mean of the differences (bias) was -0.16 l/min/m 2 , but with a standard deviation (precision) of ± 1.16 l/min/m 2 . The lower and upper limits of agreement were -2.48 and 2.16 l/min/m 2 respectively. Bland–Altman analysis of individual patients showed bias measurements of -1.31, -0.95, 0.28, 0.59, 0.88, 0.76 and -1.32 l/min/m 2 , with precision of 0.59, 0.63, 0.59, 0.61, 1.10, 0.60 and 0.74 l/min/m 2 , respectively. Three of the patients showed a poor precision throughout the measured range of cardiac output. The other four patients showed a fair degree of precision, but with a changing bias, from negative to positive, with increasing cardiac output. There were no clear factors which could be used to predict which group individual patients would fall into.

Figure 1. Scatter plot of Bomed bioimpedance vs Vigilance thermodilution continuous measurement of cardiac index ($r^2 = 0.01$).

Figure 2. Bland–Altman plot of difference in cardiac index measured by bioimpedance cardiography (bi) and continuous thermodilution (td) against mean measured cardiac index (Cl; l/min/m 2). The degree of bias (measured by the mean) and precision ($\pm 2SD$) are shown.

Discussion

The morbidity and mortality associated with the use of PACs is well recognized and must be weighed against the potential benefits for the individual patient of gaining valuable information regarding the cardiovascular system and oxygen delivery [6]. Bioimpedance cardiography offers an apparently attractive noninvasive way of estimating cardiac output and obtaining derived haemodynamic parameters. However, this is of no benefit if the information acquired is unreliable, leading to inappropriate management. We investigated the use of bioimpedance in the critically ill to assess whether it can be a reliable method of cardiac output measurement for this group of patients.

The thermodilution method is an indirect measure of cardiac output, but has been shown to correlate well with the gold standard dye dilution method. Moreover, it is the method most commonly used to measure cardiac output in patients in the ICU and upon which much of our understanding of the cardiovascular changes in critical illnesses is based.

Bioimpedance cardiography has been validated in some patient groups [7], and newer systems with improved software for advanced signal processing may be valid in critically ill patients [8]. However, there have been concerns raised as to its accuracy and reliability in ICU patients [9,10]. Correct placement of the eight electrodes is important in obtaining accurate

information; in ICU patients this may be hampered by dressing covering internal jugular line sites, thoracotomy wounds and chest drain sites. In this study, the directions for electrode placement detailed on the NCCOM 3 monitor were followed as a closely as practically possible, aiming to reproduce the conditions that would pertain to routine clinical use of the monitor.

The use of positive pressure ventilation with PEEP, and the presence of endotracheal tubes, chest drains and sternal wires may affect bioimpedance measurements by affecting the rate of change of thoracic impedance [11]. However, cardiological studies in patients with pacemakers have shown bioimpedance to be a useful technique [12]. The presence of the thermal filament in the modified PAC used by the Vigilance monitor may also affect bioimpedance measurements; standard PACs may also affect measurements by the presence of the thermistor wire. In any case, if the bioimpedance data are affected by foreign material in the thorax, this makes the use of the Bomed in ICU patients very problematic.

The design of this study is novel in two ways, giving major advantages over previous studies. Firstly, by comparing two 'continuous' methods of cardiac output measurement, the inevitable errors of synchronization using intermittent methods are virtually eliminated. (Previous studies comparing bioimpedance with thermodilution have needed to average several bolus measurements before or after the acquisition of bioimpedance data.) Secondly, we were able to collect a very large number of simultaneous paired data points from the two methods, averaged over the whole respiratory cycle, enabling a more accurate comparison.

This study shows there is essentially no relationship between cardiac index as measured by the Bomed NCCOM 3 and the continuous thermodilution method ($r^2 = 0.01$); this is surprising as the two methods claim to measure the same variable. There is extremely poor agreement between the methods according to the Bland-Altman method. The lack of precision is quite unacceptable. From our data, a cardiac index of 4.0 l/min/m² would be subject to an error of up to +54% or -62% at the 95% limits of agreement. Importantly, we were able to assess the changes in measured cardiac output in individual patients and these also showed poor agreement with variable precision and bias. This would indicate that the use of the NCCOM 3 is unlikely to be of value even if a subgroup of patients, in whom the precision is acceptable, could be identified.

The two methods cannot therefore be used interchangeably to monitor cardiac output. Furthermore, therapeutic interventions to improve cardiovascular function that have been shown to be beneficial in patients monitored by the thermodilution technique cannot be similarly applied to patients monitored by bioimpedance cardiography. Bioimpedance cardiography cannot be recommended for use in critically ill patients such as this group from a general ICU.

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Thoracic electrical bioimpedance theory and clinical possibilities in perioperative medicine

PREDRAG STEVANović • RADISAV ŠĆEPANOViĆ •
DRAGAN RADOVANOViĆ • ĐORĐE BAJEC • RADOŠLAV PERUNOViĆ •
DRAGOŠ STOJANOViĆ • DEJAN STEVANoviĆ

PREDRAG STEVANoviĆ (✉) •
RADISAV ŠĆEPANOViĆ •
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ĐORĐE BAJEC •
RADOŠLAV PERUNOViĆ •
DRAGOŠ STOJANOViĆ •
DEJAN STEVANoviĆ

Department of Anesthesiology
and Reumatology
University Clinical Hospital "Dr. D. Mišović"
11000 Belgrade, Serbia
Phone/Fax: +381 11 26 61 939
E-mail: batica@eunet.yu

ABSTRACT

This article is a short review of thoracic electrical bioimpedance (TEB) theory and clinical capabilities. Cardiac output measurement is used primarily to guide therapy in complex, critically ill patients. Thoracic electrical bioimpedance is one of several noninvasive techniques that have been investigated to measure cardiac output and other hemodynamic parameters. Opinions in current literature continue to be conflicting as to the utility of thoracic electrical bioimpedance to that purpose. There is a limited number of good designed studies but they imply TEB is an accurate and reliable noninvasive method for determining cardiac output/cardiac index and it would be valuable for patients and circumstances in which intracardiac pressures and mixed venous blood samples are not necessary.

Key words: bioimpedance, non-invasive hemodynamic monitoring, cardiac output, pulmonary artery catheter, thermo dilution

There are many high-risk patients who need the measurement of cardiac output (CO) in the operation theatre and postoperative settings. (1,2) It is still at present a dilemma about invasive and noninvasive way of monitoring hemodynamic function.

Invasive cardiac monitoring measurement is used primarily to guide therapy in complex, critically ill patients, and during the perioperative period in patients with high morbidity and mortality risk. Invasive pulmonary artery catheterization has been the method of choice for the accurate evaluation of hemodynamic status.

The pulmonary artery catheter (PAC) is routinely used to measure cardiac output by thermo dilution (TD) method

for patients undergoing coronary artery bypass surgery, but there are a large number of patients who undergo minor or minimally invasive surgery, who presented with severe cardiac disease. Patients in the intensive care unit who suffer from sepsis and great hemodynamic disturbance also need comprehensive cardiac monitoring. (3) However, we can truly ask ourselves: do we really need Swan-Ganz during a laparoscopic cholecystectomy if a cardiac condition is quite bad? There are many similar situations in everyday practice.

It is widely recognized that the pulmonary artery catheter (PAC) places the patient at risk for infection and many other complications. (4) That possibility and lack of Swan-Ganz experience in a majority of anaesthetists, force a constant effort to find good, reliable and accurate noninvasive hemodynamic monitoring.

When we speak about hemodynamic monitoring, at first we think about cardiac output. There is no totally accurate method of measuring cardiac output, but it can be estimated on the basis of various assumptions. (5) CO per se means not much if we do not put it in relation with adequacy of oxygen transport, which is the ultimate goal of hemodynamic monitoring. Oxygen transport is a function of CO and arterial oxygen content.

Cardiac output (CO) is assessed by measuring the volume of blood pumped by the heart in one minute. The amount of blood pumped by the left ventricle in one contraction is the stroke volume. The stroke volume (SV) multiplied by the heart rate (HR) is the cardiac output:

$$CO = SV \cdot HR$$

CO is directly proportional to the mean pressure drop over the whole of the

systemic arterial-venous circuit (i.e., mean arterial pressure (MAP) – central venous pressure (CVP)) and is inversely proportional to the total peripheral resistance (TPR) of the circuit.

$$CO \approx (MAP - CVP) / TPR$$

There is an analogous relationship in electricity. Ohm's Law states that flow of current (I , analogous to CO) is equal to the voltage drop (V , analogous to the pressure drop) between two ends of a circuit, divided by the resistance to current flow (R , analogous to TPR). For direct current we can state:

$$I = V / R$$

$$R = V / I$$

If $I = \text{constant}$, a change in resistance (ΔR) is proportional to a change in voltage (ΔV):

$$\Delta R \approx \Delta V$$

For alternating current flow, resistance is known as impedance (Z) and is complex, frequency-dependent parameter. For alternating current we can say that:

$$Z = V / I$$

Z is measured in ohms. If I remains constant, then periodic changes in voltage (ΔV) produce concurrent changes in impedance (ΔZ):

$$\Delta Z \approx \Delta V$$

This simple equation is fundamental to understanding SV determination by thoracic electrical bioimpedance (TEB). Thoracic Electrical Bioimpedance (TEB), with a symbol Z , is an electrical resistance of the thorax to a high-frequency, very-low magnitude TEB measurement current. TEB utilizes a patient's thorax as an impedance transducer.

The impedance to alternating current flow of a simple cylindrical electrical conductor is equal to its specific resistance (ρ) times its length (L), divided by its cross-sectional area (A):

$$Z = \rho L / A$$

For simplicity, the thorax can be modeled as a cylindrical conductor of length L , which has embedded, in parallel with the thoracic length, a smaller cylindrical conductor, representing the great vessels (figure 1). In that small cylinder representing the vessels is blood of specific resistance ρ_b , with cross-sec-

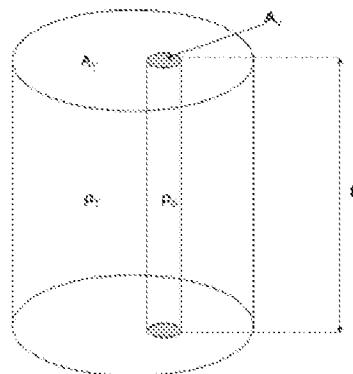


Figure 1. Simple model of human thorax. Small cylinder represents great vessels with cross-sectional area Av , length L and specific resistance of blood ρ_b . Remaining thoracic volume has cross-sectional area AT , length L and specific resistance ρ_T ($\rho_b \ll \rho_T$).

tional area labeled Av . The remaining volume of the cylinder is assumed to be homogenous with specific resistance ρ_T (ρ thorax) and cross-sectional area labeled AT .

It is assumed that the small cylinder has a varying cylindrical cross-sectional area: $Av + \Delta Av(t)$, caused by aortic and pulmonary artery pulsations with each heart beat. If we assumed that $\rho_b \ll \rho_T$ we can conclude that the change in Z with each heart beat is caused mostly by changes in the cross-sectional areas of the great vessels $\Delta Av(t)$. Synthesizing this theory with Ohm's Law, it comes about that the periodic increases in cross-sectional area (L remaining constant) and volume of the great vessels (ΔAv) must cause corresponding decreases in thoracic impedance and vice versa.

Systemic aortic blood pressure and expansion of the great vessels are accompanied by simultaneous chan-

ges in thoracic impedance (ΔZ). The increased thoracic conductivity is caused by the systolic pumping of blood into the great vessels from the ventricles, caused by ventricular systole, and it is registered as decreases in impedance to current flow.

Time-varying pulsatile changes in aortic and pulmonic blood volume (ΔV) are directly proportional to the time-varying, cardiac-induced impedance change (ΔZ):

$$\Delta V (t) \approx \rho_b (L^2 / Z_0^2) \Delta Z ; \text{ where } Z_0 \text{ is the nonpulsatile base impedance measured.}$$

The Base Impedance (Z_0) is indirectly proportional to total content of thoracic fluids. We cannot identify individual conductance contributions of the intravascular, intra-alveolar and interstitial compartments and therefore, we can see the thoracic fluid conductivity, which is then directly proportional to the thoracic fluids content (TFC). The TEB variations and changes (ΔZ) are produced by:

- slow changes of fluid levels in all thoracic compartments,
- tidal changes of venous and pulmonary blood volume caused by respiration,

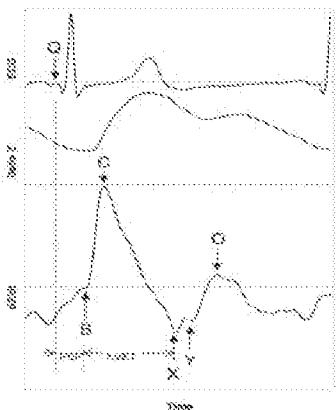


Figure 2. ECG and ICG Waveforms.
Q = Ventricular depolarization, **S** = Opening aortic and pulmonic valves, **C** = Maximal slope ΔZ , **X** = Closure aortic valve, **Y** = closure of pulmonic valve, **O** = Opening mitral valve / rapid filling of ventricles.

- volumetric and velocity (alignment of planes of erythrocytes as a function of blood velocity) changes of aortic blood produced by the heart's pumping activity.

The cardiac origin of ΔZ is obvious when viewed in light of its electrocardiogram (ECG) counterpart (figure 2). Like ECG, TEB has important waveform characteristics that reflect significant points in the cardiac cycle (figure 3). The change in impedance (ΔZ) is measured from the baseline impedance Z_0 (shown as dotted line). Baseline impedance is inversely proportional to the amount of conductive material in the thorax. The thoracic fluid content parameter (TFC) is the inverse of baseline impedance, so TFC is directly proportional to the amount of conductive material in the thorax. As fluid in the chest increases,

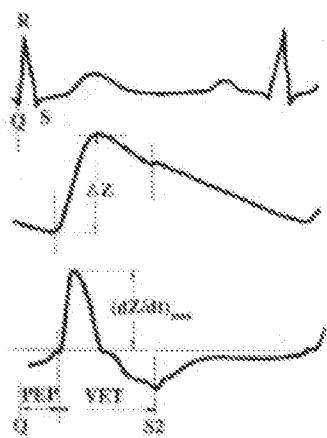


Figure 3. The timing relationship between ECG and thoracic electrical bioimpedance: myocardial contraction starts at the Q-time of the ECG QRS complex. The Pre-Ejection Period (PEP) (isovolumic contraction) is defined as the elapsed time between the Q-time of the QRS complex and the opening of aortic valve. The ejection phase, outlined by the Ventricular Ejection Time (VET), starts by opening of aortic valve and ends by its closure (S2-time). During the initial portion of ejection phase the aorta distends and the thorax, therefore, becomes more conductive; at the same time the velocity of blood increases, more erythrocytes are aligned so their planes are parallel with the main axis of aorta and, therefore, the blood becomes more conductive.

TFC increases. The first derivative (dz/dt) of the delta Z waveform is used to identify the maximum upstroke, shown as point C. This is used to calculate Velocity Index (VI), which is indicative of aortic blood velocity. As the heart pumping ability is impaired, VI decreases. The left ventricular ejection time (LVET) is the time from the opening of the aortic valve (B point) to the closing (X point). As the heart loses its ability to contract (systolic function worsens), LVET shortens.

The patient is connected to the monitor via a patient cable attached to eight solid-gel, disposable electrodes (figure 4). The TEB measurement current is passed through the thorax in a direction parallel with the spine between the beginning of the thorax (the line at the root of the neck) and the end of the thorax (at the level of diaphragm - the xiphoid process level). Four dual sensors with eight lead wires are placed on neck and chest. Current transmitted by outer electrodes seek the path of least resistance: blood filled aorta. Baseline impedance (resistance) is measured using inner electrodes. With each heartbeat, blood volume and velocity in the aorta changes. Corresponding change in impedance is measured. Baseline and changes in impedance are used to measure and calculate hemodynamic parameters.

These four electrodes also detect four different vectors of the ECG signal. The Heart Rate (HR) is derived from the R-R intervals of the ECG signal. Due to the anatomical shape of the thorax, a preferential placement for all eight electrodes is along the frontal plane - the widest thoracic dimension.

With TEB we can get many noninvasive parameters (table 1).

From a clinical point of view, we can divide all this parameters into four groups (table 2).

We can use TEB for hemodynamic evaluation in many circumstances:

- Fluid management for heart failure,
- Differentiation of cardiogenic from pulmonary causes of acute dyspnea,
- Optimization of atrioventricular interval in cardiac pacemakers,

- Monitoring of patients for early diagnosis of rejection after heart transplantation,
- Management of drug-resistant hypertension,
- Evaluation of hemodynamic response in dehydrated patients,
- Management of patients with severe cardiac illness during surgery,
- Management of patients during intensive care,
- Management of patients in the Emergency Department,
- Noninvasive monitoring for early recognition and treatment of shock in high-risk trauma and surgical patients.

In the perioperative fluid management, which is everyday practice, thoracic fluid content (TFC) could be very applicable parameter.

- A single TFC measurement indicates total conductivity of the chest that is affected by both intravascular and extra vascular fluid.
- Changes in TFC from a previous measurement represent changes in total fluid of the chest, intravascular and/or extra vascular volume.

Although TFC does not correlate with PCWP, it is a very good indicator of thoracic fluid. Changes in TFC are very reliable indicators of changes in intra- or extra-vascular fluid volume.

There are many decompensated heart failure patients preoperative and in the intensive care unit. It is impossible to imagine that all of them are candidates for invasive monitoring. There is a place for TEB in the differentiation of cardiogenic from pulmonary causes of acute dyspnea when history, physical examination, and standard assessment tools provide insufficient information. Some authors investigate usefulness of the impedance value on a scale of 1 to 10 for each heart failure patient. (6) The mean usefulness rating was 7.9 (95% Confidence Interval: 6.5 – 9.3). The researchers concluded that the baseline impedance value obtained from noninvasive hemodynamic monitoring with TEB accurately predicted the presence and severity of pulmonary

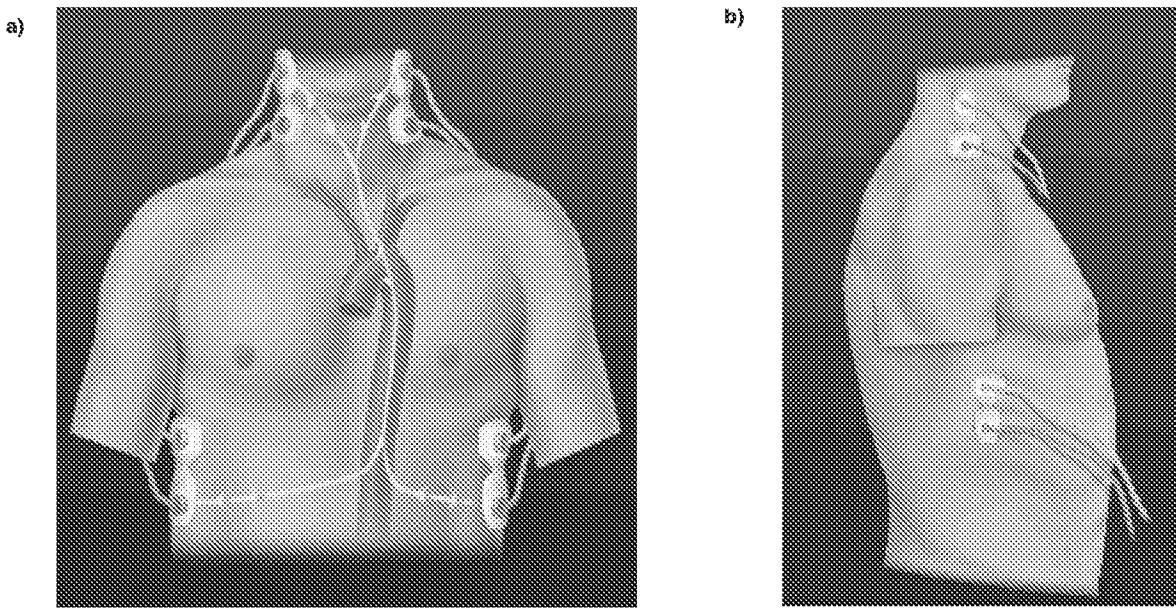


Figure 4. Location of the 8 electrodes along the TEB transducer - a patient's thorax. The top and bottom pair is a source and sink of the TEB measurement current, the inner pairs, located at the root of the neck (the beginning of the transducer) and the diaphragm level, i.e., the xiphoid process level (the end of the transducer), are used for sensing both the TEB signal and 4 different vectors of the ECG signal. a) Front view b) Lateral view

edema and can serve as an important adjunct in the treatment of heart failure patients. In many operative procedures TEB may be used instead of invasive techniques as it is accurate, reliable and simple to use, with proven efficacy. (7) It has the additional advantage of allowing continuous monitoring of cardiac index by noninvasive means, and gives the anesthetist information regarding stroke volume, cardiac and ejection velocity indices in a "real-time" manner, in response to drugs or surgical stimuli. It is well known that respiration and artificial ventilation result in cyclic fluctuations of the cardiac output determination by thermodilution (TD). Because of the modulation shift during changing ventilation patterns, i.e. different levels of PEEP, researchers doubt that the TD technique is an appropriate method for studying modulations of cardiac output dependent on artificial ventilation. Castor and colleagues in their study find that TEB is an accurate and reproducible method for determination of cardiac output under these changing respiratory patterns during artificial ventilation. (8)

There are many studies that compare TEB and direct Fick cardiac output methods. In a prospective study, Van DeWater et al. (9) compared the accuracy of TEB to thermodilution (CO-TD) in postoperative coronary artery bypass graft (CABG) patients. This study took place in a cardiovascular-thoracic surgery intensive care unit (ICU). The study included 53 post-CABG patients from whom 210 pairs of cardiac output measurements were made. The TEB cardiac output was determined simultaneously with the TD cardiac output. The authors reported that when comparing TEB to TD, they found that the bias, precision, correlation slope, and intercept were equivalent to TD. The authors stated that in those circumstances in which intracardiac pressures and mixed venous blood samples are not necessary, TEB is preferable to invasive TD method in determining cardiac output. The authors stated that the TEB monitor allows for quick and easy cardiac output monitoring and systemic vascular resistance in clinical areas where the pulmonary artery catheter is not typically utilized (e.g., emergency

department, sub acute care, hypertension and heart failure). "The latest TEB technology for determining CO is less variable and more reproducible in an intrapatient sense than is CO-TD." TEB is reproducible, especially in comparison to serial measurements using Thermo dilution. The historical standard for hemodynamic measurement, thermo dilution (TD), shows only modest correlation when compared to itself. The SD for thermodilution is about 1 liter/minute or about 20% of the average CO. TEB shows very high correlation when compared to itself, and lower standard deviation for multiple TEB measurements.

In the other study of 23 adults in the intraoperative and post operative settings, the correlation coefficient between the two methods was $r=0.89$, $p<0.001$. (10) In a study of 68 critically ill patients, changes in cardiac output estimated by TEB were found to closely correlate with values obtained with TD method $r = 0.86$, $p<0.001$. (11)

Shock and shock-related organ failure account for most deaths in trauma and surgical patients. The study of Asensio

and colleges examines the use of non-invasive methods, including thoracic electrical bioimpedance (TEB) for early recognition and treatment of shock in high-risk trauma and surgical patients. (12) This study involved a series of trauma patients entering the emergency department. It reviews the use of invasive and noninvasive monitoring methods in these patients to describe the temporal patterns of cardiac, pulmonary, and tissue perfusion functions in the early phase of trauma. The authors concluded that noninvasive monitoring is easy to apply, safe, inexpensive, and more cost-effective than invasive monitoring. High-risk patients might profit by earlier noninvasive hemodynamic monitoring. If noninvasive monitoring identifies circulatory problems earlier, then even more expeditious therapy could be given to achieve optimal physiologic goals that improve outcome.

Shoemaker and colleges in their study evaluate the feasibility of multicomponent hemodynamic monitoring in critical emergency patients and compares the technique of TEB with simultaneous monitoring by the pulmonary artery thermodilution catheter. (13) The researchers concluded that, "Noninvasive monitoring can provide hemodynamic and perfusion information previously available by invasive thermodilution catheters. Such noninvasive monitoring can display continuous on-line real-time data, allowing immediate recognition of circulatory abnormalities and providing a means to titrate therapy to appropriate therapeutic goals."

Potential limitations in use of TEB are states with HR > 250 bpm, septic shock (end stage sepsis), severe aortic valve regurgitation, extremely high blood pressure (MAP > 130) and intra-aortic balloon pump. Also, extremely tall (>210 cm) and low (<120 cm) patients and patients under 35 kg and over 170 kg lose out the accuracy of measurement by TEB.

Conclusion

Thoracic electrical bioimpedance is one of several noninvasive techniques that have been used to measure cardiac

Table 1. Parameters captured by TEB measurement.

Parameter	Range (Normal Values)
Cardiac Output	0-30 L/min (Variable Normal Values)
Cardiac index	0-15 L/min/m ² (2.5 – 4.7 L/min/m ²)
Stroke Volume	0-250 ml (Variable Normal Values)
Stroke Index	0-125 ml/m ² (35-65 ml/m ²)
Systemic Vascular Resistance	0-5000 dyne-sec-cm ⁻⁵ (742-1378 dyne-sec-cm ⁻⁵)
Systemic Vascular Resistance Index	0-10,000 dyne-sec-cm ⁻⁵ -m ² (1337-2483 dyne-sec-cm ⁻⁵ -m ²)
Thoracic Fluid Content	10-150 /kΩ (30-60 /kΩ - males 21-37 /kΩ - females)
Pre-ejection Period	0-1000 msec (Variable Normal Values)
Acceleration Index	1-400 /100s ² (70-150 /100s ² – males 90-170 /100s ² – females)
LV Ejection Time	0-1500 msec (Variable Normal Values)
Velocity Index	0-200/1000s (33-65/1000s)
Systolic Time Ratio	0-1.0 (0.3 – 0.5)
Left Stroke Work Index	0-200 gm-m/m ² (51.6-74.3 gm-m/m ²)
Indexed Left Cardiac Work	0-25 kg-m/m ² (3.0-5.5 kg-m/m ²)
Heart Rate	40-250 beats/min (58-86 beats/min)
Estimated Delivered O ₂ Index	0-2000 ml/min/m ² (550-650 ml/min/m ²)

output and other hemodynamic parameters. It is a simple, cost-effective tool for clinical assessment. TEB repeatedly demonstrated good correlation with other clinical measures of cardiac function and associated parameters. The advancements in hardware, enhanced algorithms used in software, and clinical experience may well expand the clinical applications for this continuous noninvasive hemodynamic monitoring. We must say that evidence in the current literature continue to be conflicting

as to the utility of TEB in measuring hemodynamic parameters but TEB has demonstrated clinically acceptable accuracy with lower cost per patient in those circumstances in which intracardiac pressures and mixed venous blood samples are not necessary. Furthermore, it enables us to quickly and easily monitor CO and SVR in clinical areas where the invasive monitoring is not typically utilized. The use of TEB may potentially improve patient outcomes.

Table 2. The hemodynamic parameters measured and calculated by TEB reflect cardiac flow (output) and the four determinants of cardiac output (HR, preload, afterload, and contractility).

Flow	Stroke Volume / Index (SV / SI) Cardiac Output / Index (CO / CI)
Resistance	Systemic Vascular Resistance (SVR) Index (SVRI)
Contractility	Systolic Time Ratio (STR) Pre-ejection Period (PEP) LV Ejection Time (LVET) Velocity Index (VI) Acceleration Index (ACI)
Fluid	Thoracic Fluid Content (TFC)

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PACEMAKER MALFUNCTION DUE TO MICROCURRENT INJECTION FROM A BIOIMPEDANCE NONINVASIVE CARDIAC OUTPUT MONITOR

J. Antonio Aldrete, MD, MS, Carol Brown, RN, Judy Daily, RN, and Valerie Buerke, BA, MS

Aldrete JA, Brown C, Daily J, Buerke V. Pacemaker malfunction due to microcurrent injection from a bioimpedance noninvasive cardiac output monitor.

J Clin Monit 1995;11:131-133

INTRODUCTION

Noninvasive cardiac output monitors (NICOMs) may yield inaccurate measurements in patients with pacemakers. However, the information included with the pacemaker and the NICOM does not mention this possibility, nor does it describe the possibility that microcurrents injected into the thoracic wall by NICOM may accelerate the pacemaker's rate to the point of rendering it ineffective. Herein we describe a case of a sudden spike-rate acceleration of a pacemaker when a NICOM was connected to a patient's chest wall on two different occasions.

CASE HISTORY

An 82-year-old man with posttraumatic degenerative disk disease and spondylolisthesis of the cervical spine was scheduled to have a cervical epidural block. The patient had a history of arteriosclerotic heart disease and three years previously had undergone a coronary artery bypass graft (CABG) and the insertion of a multiprogrammable, minute ventilation, rate-responsive pulse generator with telemetry (Teletronic Pacing Systems, Englewood, CO). For 11 months, the pacemaker had functioned well.

In the treatment room, a monitor (Criticor, Dimamap Plus, Vital Signs), was attached to the patient, obtaining a control electrocardiograph (ECG) tracing (Fig 1), blood pressure (BP) = 134/60, heart rate (HR) = 78, SpO_2 = 96%. As part of a study on the hemodynamic effects of cervical epidural blocks, pre-gelled electrodes of a NICOM (BoMed NICOM3-R7, Irvine, CA) were connected as follows: two on the neck along the carotid artery, two on the lateral aspect of the chest at the level of the xiphoid bilaterally, and one bipolar electrode on the anterior chest wall (Fig 2) to receive the outgoing signals. The NICOM was activated at 8:24. After the completion of the data entry process, it was noted in the ECG that the heart rate suddenly increased to 120 beats/min, with blood pressure = 134/71, SpO_2 = 96% at 8:29. Monitoring was continued throughout. The patient was treated with nasal oxygen 3 L/min. At 8:40, lidocaine 50 mg intravenously was given and repeated 2 min later. The heart rate remained unchanged, even after 15 mg of esmolol HCP was given, at divided dosages, within the next 10 min. At 8:45, the vital signs were: blood pressure = 143/73,

From the Center for Pain Management, Destin, FL.

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Address correspondence to Dr Aldrete, Center for Pain Management, 807 Melvin Street, Destin, FL 32541.

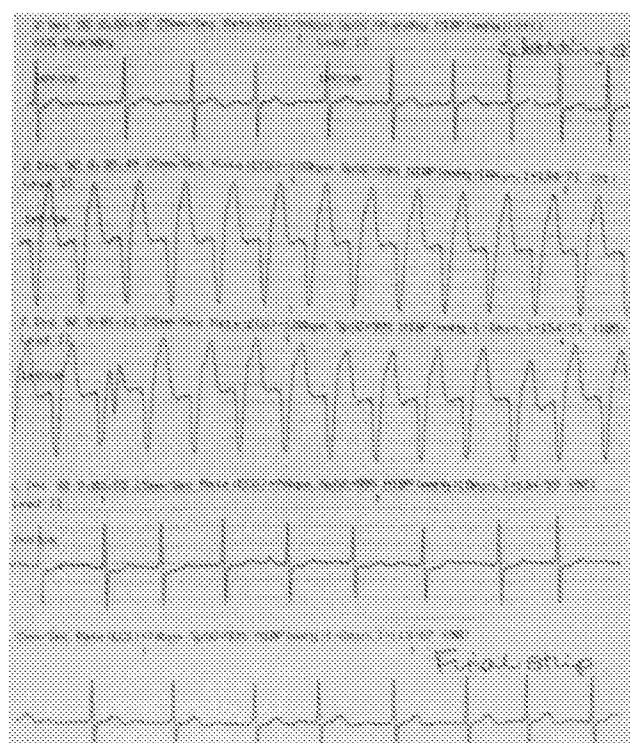


Fig 1. Patient's electrocardiograph tracing.

heart rate = 120 beats/min, SpO_2 = 98%. No significant change was noted. At 8:55, 0.25 mg of neostigmine was administered intravenously without obvious change. At 8:59 the NICOM was discontinued. By 9:03, the vital signs were: blood pressure = 154/71, heart rate = 76 beats/min, SpO_2 = 99%. A *posteriori*, a consulting cardiologist read the ECG tracings as a malfunctioning pacemaker with spikes predominating at a fixed rate of 120/min. Two hours later, the cardiologist failed to find any alteration of the pacemaker's function or any worsening of the patient's cardiovascular condition.

Two weeks later, as part of another study, the same NICOM was attached. The monitor obtained, as basic readings, blood pressure = 146/92, heart rate = 76 beats/min, SpO_2 = 92%, and a control tracing of a bipolar ECG. After placement of the electrodes, when the NICOM was turned on, the patient again exhibited a heart rate of 120 beats/min, blood pressure of 138/82, and SpO_2 94%. The ECG tracing was similar to that observed previously (consisting of pacemaker spikes). The onset of tachycardia coincided with the time when the NICOM was activated.

The predominance of spike action again suggested a pacemaker malfunction triggered by the NICOM. This time, the heart rate spontaneously returned to normal

within 4 min of disconnecting the machine. Throughout this period, the patient remained alert and comfortable, with the remainder of the vital signs within normal limits.

DISCUSSION

The measurement of cardiac output by thoracic bioimpedance is based on the principle that a pulsatile change in resistance is generated as a 2.5-mA current is injected through surface gel electrodes. These electrodes are placed on the neck and lateral aspect of the thoracic wall (see Fig 2). Supposedly, the outer pairs of electrodes inject a 70-R Hz, 2.5-mA current, which is then perceived by the inner pairs of electrodes [1]. The resistance to the injected high-frequency current is dependent upon the fluid characteristics of the thoracic volume. These changes are then timed to the ventricular electrical depolarization and mechanical systole, thus establishing a quantitative correlation of blood flow changes and ultimately stroke volume, from which cardiac output and ejection fraction can be derived [2,3].

The limitations of this technology have been docu-

Voltage Sensing Electrodes

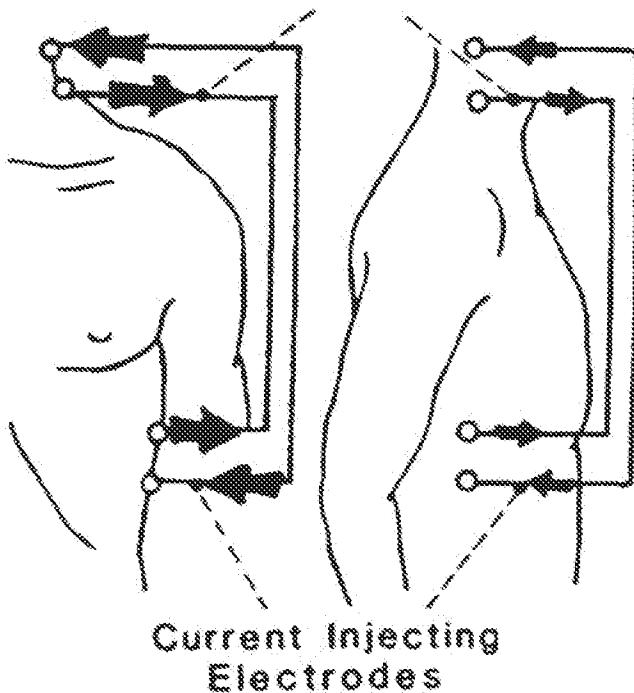


Fig 2. Pre-gelled electrodes were connected to the patient as follows: two on the neck along the carotid artery; two on the lateral aspect of the chest at the level of the xiphoid bilaterally; and one bipolar electrode on the anterior chest wall.

mented previously [1,4]. The measurements are considered less accurate in severe dysrhythmias and tachycardia, in which pacemaker spikes result in incorrect determination of systolic time intervals, heart-rate determination, and, therefore, inaccurate cardiac output measurements [4,5]. Signal acquisition is impaired by electrode adherence defects and electrocautery [6]. The manufacturer's information included with the NICOM does not specifically warn about the possibility of its affecting pacemakers [2]. Although the alternate current delivered by bioimpedance monitors to measure thoracic resistance has been termed as "microcurrent," it appears that it is large enough to alter the performance of pacemakers. Though we failed to recognize the hazardous association of the two devices after the first incident, after the second occurrence we identified the link between them that prompted the report.

The "operator's manual" and "patient brochure" [7] of this type of pacemaker list only the following as possible dangers of interference:

1. all electrical appliances
2. microwave-operated devices
3. close proximity of electrical motors, generators, welders, and transmitters

Pacemaker manufacturers may wish to add NICOM-R7 to the list of potential hazards, while manufacturers of bioimpedance NICOMs need to list it as potentially dangerous to patients with cardiac pacemakers.

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In re Patrick H. O'FARRELL, Barry A. Polisky and David H. Gelfand.

No. 87-1486.

**United States Court of Appeals,
Federal Circuit.**

Aug. 10, 1988.

J. Bruce McCubbrey, Fitch, Even, Tabin & Flannery, of San Francisco, Cal., argued for appellant. Virginia H. Meyer, Fitch, Even, Tabin & Flannery, of San Francisco, Cal., was on the brief for appellant.

Harris A. Pitlick, Associate Sol., of Arlington, Va., argued for appellee. With him on the brief were Joseph F. Nakamura, Sol. and Fred E. McKelvey, Deputy Sol.

Before MARKEY, Chief Judge, and RICH and NIES, Circuit Judges.

RICH, Circuit Judge.

1 This appeal is from the decision of the United States Patent and Trademark Office Board of Patent Appeals and Interferences (board) affirming the patent examiner's final rejection of patent application Serial No. 180,424, entitled "Method and Hybrid Vector for Regulating Translation of Heterologous DNA in Bacteria." The application was rejected under 35 U.S.C. Sec. 103 on the ground that the claimed invention would have been obvious at the time the invention was made in view of a published paper by two of the three coinventors, and a publication by Bahl, Marians & Wu, 1 Gene 81 (1976) (Bahl). We affirm.

2 The claimed invention is from the developing new field of genetic engineering. A broad claim on appeal reads:

3 Claim 1. A method for producing a predetermined protein in a stable form in a transformed host species of bacteria comprising, providing a cloning vector which includes at least a substantial portion of a gene which is indigenous to the host species of bacteria and is functionally transcribed and translated in that species, said substantial portion of said indigenous gene further including the regulatory DNA sequences for RNA synthesis and protein synthesis but lacking the normal gene termination signal, and linking a natural or synthetic heterologous gene encoding said predetermined protein to said indigenous gene portion at its distal end, said heterologous gene being in proper orientation and having codons arranged in the same reading frame as the codons of said indigenous gene portion so that readthrough can occur from said indigenous gene portion into said heterologous gene in the same reading frame, said heterologous gene portion further containing sufficient DNA sequences to result in expression of a fused protein having sufficient size so as to confer stability on said predetermined protein when said vector is used to transform said host species of bacteria.

4 Illustrative embodiments are defined in more specific claims. For example:

5 Claim 2. A method for producing a predetermined protein in a stable form in a transformed host species of bacteria, comprising, providing an E. coli plasmid having an

« up r operator, a promoter, a site for the initiation of translation, and at least a substantial portion of the beta-galactosidase gene of the *E. coli* lactose operon, said substantial portion of said beta-galactosidase gene being under the control of said operator, promoter and site for initiation of translation, said substantial portion of said beta-galactosidase gene lacking the normal gene termination signal, and linking a heterologous gene encoding said predetermined protein to said beta-galactosidase gene portion at its distal end, said heterologous gene being in proper orientation and having codons arranged in the same reading frame as the codons of the said beta-galactosidase gene portion so that readthrough can occur from said beta-galactosidase gene portion into said heterologous gene in the same reading frame, said heterologous gene portion further containing sufficient DNA sequences to result in expression of a fused protein having sufficient size so as to confer stability on said predetermined protein when said vector is used to transform said host species of bacteria.

6 Claim 3. The method of Claim 2 wherein said *E. coli* plasmid comprises the plasmid designated pBGP120.

7 Although the terms in these claims would be familiar to those of ordinary skill in genetic engineering, they employ a bewildering vocabulary new to those who are not versed in molecular biology. An understanding of the science and technology on which these claims are based is essential before one can analyze and explain whether the claimed invention would have been obvious in light of the prior art.

I. Background¹

8 Proteins are biological molecules of enormous importance. Proteins include enzymes that catalyze biochemical reactions, major structural materials of the animal body, and many hormones. Numerous patents and applications for patents in the field of biotechnology involve specific proteins or methods for making and using proteins. Many valuable proteins occur in nature only in minute quantities, or are difficult to purify from natural sources. Therefore, a goal of many biotechnology projects, including appellants' claimed invention, is to devise methods to synthesize useful quantities of specific proteins by controlling the mechanism by which living cells make proteins.

9 The basic organization of all proteins is the same. Proteins are large polymeric molecules consisting of chains of smaller building blocks, called amino acids, that are linked together covalently.² The chemical bonds linking amino acids together are called peptide bonds, so proteins are also called polypeptides.³ It is the exact sequence in which the amino acids are strung together in a polypeptide chain that determines the identity of a protein and its chemical characteristics.⁴ Although there are only 20 amino acids, they are strung together in different orders to produce the hundreds of thousands of proteins found in nature.

10 To make a protein molecule, a cell needs information about the sequence in which the amino acids must be assembled. The cell uses a long polymeric molecule, DNA (deoxyribonucleic acid), to store this information. The subunits of the DNA chain are called nucleotides. A nucleotide consists of a nitrogen-containing ring compound (called a base) linked to a 5-carbon sugar that has a phosphate group attached.⁵ DNA is composed of only four nucleotides. They differ from each other in the base region of the molecule. The four bases of these subunits are adenine, guanine, cytosine, and thymine (abbreviated respectively as A, G, C and T). The sequence of these bases along the DNA molecule specifies which amino acids will be inserted in sequence into the polypeptide chain of a protein.

11 DNA molecules do not participate directly in the synthesis of proteins. DNA acts as a

« up Permanent "blueprint" of all of the genetic information in the cell, and exists mainly in extremely long strands (called chromosomes) containing information coding for the sequences of many proteins, most of which are not being synthesized at any particular moment. The region of DNA on the chromosome that codes for the sequence of a single polypeptide is called a gene.⁶ In order to express a gene (the process whereby the information in a gene is used to synthesize new protein), a copy of the gene is first made as a molecule of RNA (ribonucleic acid).

12 RNA is a molecule that closely resembles DNA. It differs, however, in that it contains a different sugar (ribose instead of deoxyribose) and the base thymine (T) of DNA is replaced in RNA by the structurally similar base, uracil (U). Making an RNA copy of DNA is called transcription. The transcribed RNA copy contains sequences of A, U, C, and G that carry the same information as the sequence of A, T, C, and G in the DNA. That RNA molecule, called messenger RNA, then moves to a location in the cell where proteins are synthesized.

13 The code whereby a sequence of nucleotides along an RNA molecule is translated into a sequence of amino acids in a protein (i.e., the "genetic code") is based on serially reading groups of three adjacent nucleotides. Each combination of three adjacent nucleotides, called a codon, specifies a particular amino acid. For example, the codon U-G-G in a messenger RNA molecule specifies that there will be a tryptophan molecule in the corresponding location in the corresponding polypeptide. The four bases A, G, C and U can be combined as triplets in 64 different ways, but there are only 20 amino acids to be coded. Thus, most amino acids are coded for by more than one codon. For example, both U-A-U and U-A-C code for tyrosine, and there are six different codons that code for leucine. There are also three codons that do not code for any amino acid (namely, U-A-A, U-G-A, and U-A-G). Like periods at the end of a sentence, these sequences signal the end of the polypeptide chain, and they are therefore called stop codons.

14 The cellular machinery involved in synthesizing proteins is quite complicated, and centers around large structures called ribosomes that bind to the messenger RNA. The ribosomes and associated molecules "read" the information in the messenger RNA molecule, literally shifting along the strand of RNA three nucleotides at a time, adding the amino acid specified by that codon to a growing polypeptide chain that is also attached to the ribosome. When a stop codon is reached, the polypeptide chain is complete and detaches from the ribosome.

15 The conversion of the information from a sequence of codons in an RNA molecule into the sequence of amino acids in a newly synthesized polypeptide is called translation. A messenger RNA molecule is typically reused to make many copies of the same protein. Synthesis of a protein is usually terminated by destroying the messenger RNA. (The information for making more of that protein remains stored in DNA in the chromosomes.)

16 The translation of messenger RNA begins at a specific sequence of nucleotides that bind the RNA to the ribosome and specify which is the first codon that is to be translated. Translation then proceeds by reading nucleotides, three at a time, until a stop codon is reached. If some error were to occur that shifts the frame in which the nucleotides are read by one or two nucleotides, all of the codons after this shift would be misread. For example, the sequence of codons [...] codes for the chain of amino acids [...] leucine-serine-valine-threonine-...]. If the reading of these groups of three nucleotides is displaced by one nucleotide, such as [...] codes for the chain of amino acids [...] serine-alanine-leucine-proline-...]. This would be an entirely different peptide, and most probably an undesirable and

« up seless one. Synthesis of a particular protein requires that the correct register or reading frame be maintained as the codons in the RNA are translated.

17 The function of messenger RNA is to carry genetic information (transcribed from DNA) to the protein synthetic machinery of a cell where its information is translated into the amino acid sequence of a protein. However, some kinds of RNA have other roles. For example, ribosomes contain several large strands of RNA that serve a structural function (ribosomal RNA). Chromosomes contain regions of DNA that code for the nucleotide sequences of structural RNAs and these sequences are transcribed to manufacture those RNAs. The DNA sequences coding for structural RNAs are still called genes even though the nucleotide sequence of the structural RNA is never translated into protein.

18 Man, other animals, plants, protozoa, and yeast are eucaryotic (or eukaryotic) organisms: their DNA is packaged in chromosomes in a special compartment of the cell, the nucleus. Bacteria (procaryotic or prokaryotic organisms) have a different organization. Their DNA, usually a circular loop, is not contained in any specialized compartment. Despite the incredible differences between them, all organisms, whether eucaryote or procaryote, whether man or mouse or lowly bacterium, use the same molecular rules to make proteins under the control of genes. In all organisms, codons in DNA are transcribed into codons in RNA which is translated on ribosomes into polypeptides according to the same genetic code. Thus, if a gene from a man is transferred into a bacterium, the bacterium can manufacture the human protein. Since most commercially valuable proteins come from man or other eucaryotes while bacteria are essentially little biochemical factories that can be grown in huge quantities, one strategy for manufacturing a desired protein (for example, insulin) is to transfer the gene coding for the protein from the eucaryotic cell where the gene normally occurs into a bacterium.

19 Bacteria containing genes from a foreign source (heterologous genes) integrated into their own genetic makeup are said to be transformed. When transformed bacteria grow and divide, the inserted heterologous genes, like all the other genes that are normally present in the bacterium (indigenous genes), are replicated and passed on to succeeding generations. One can produce large quantities of transformed bacteria that contain transplanted heterologous genes. The process of making large quantities of identical copies of a gene (or other fragment of DNA) by introducing it into procaryotic cells and then growing those cells is called cloning the gene. After growing sufficient quantities of the transformed bacteria, the biotechnologist must induce the transformed bacteria to express the cloned gene and make useful quantities of the protein. This is the purpose of the claimed invention.

20 In order to make a selected protein by expressing its cloned gene in bacteria, several technical hurdles must be overcome. First the gene coding for the specific protein must be isolated for cloning. This is a formidable task, but recombinant DNA technology has armed the genetic engineer with a variety of techniques to accomplish it.⁷ Next the isolated gene must be introduced into the host bacterium. This can be done by incorporating the gene into a cloning vector. A cloning vector is a piece of DNA that can be introduced into bacteria and will then replicate itself as the bacterial cells grow and divide. Bacteriophage (viruses that infect bacteria) can be used as cloning vectors, but plasmids were the type used by appellants. A plasmid is a small circular loop of DNA found in bacteria, separate from the chromosome, that replicates like a chromosome. It is like a tiny auxilliary chromosome containing only a few genes. Because of their small size, plasmids are convenient for the molecular biologist to isolate and work with. Recombinant DNA technology can be used to modify plasmids by splicing in cloned eucaryotic genes and other useful segments of DNA containing control sequences. Short

« up pieces of DNA can even be designed to have desired nucleotide sequences, synthesized chemically, and spliced into the plasmid. One use of such chemically synthesized linkers is to insure that the inserted gene has the same reading frame as the rest of the plasmid; this is a teaching of the Bahl reference cited against appellants. A plasmid constructed by the molecular geneticist can be inserted into bacteria, where it replicates as the bacteria grow.

21 Even after a cloned heterologous gene has been successfully inserted into bacteria using a plasmid as a cloning vector, and replicates as the bacteria grow, there is no guarantee that the gene will be expressed, i.e., transcribed and translated into protein. A bacterium such as *E. coli* (the species of bacterium used by appellants) has genes for several thousand proteins. At any given moment many of those genes are not expressed at all. The genetic engineer needs a method to "turn on" the cloned gene and force it to be expressed. This is the problem appellants worked to solve.

II. Prior art

22 Appellants sought to control the expression of cloned heterologous genes inserted into bacteria. They reported the results of their early efforts in a publication, the three authors of which included two of the three coinventor-appellants (the Polisky reference⁸), that is undisputed prior art against them. Their strategy was to link the foreign gene to a highly regulated indigenous gene. Turning on expression of the indigenous gene by normal control mechanisms of the host would cause expression of the linked heterologous gene.

23 As a controllable indigenous gene, the researchers chose a gene in the bacterium *E. coli* that makes beta-galactosidase. Beta-galactosidase is an enzyme needed to digest the sugar, lactose (milk sugar). When *E. coli* grows in a medium that contains no lactose, it does not make beta-galactosidase. If lactose is added to the medium, the gene coding for beta-galactosidase is expressed. The bacterial cell makes beta-galactosidase and is then able to use lactose as a food source. When lactose is no longer available, the cell again stops expressing the gene for beta galactosidase.

24 The molecular mechanisms through which the presence of lactose turns on expression of the beta-galactosidase gene has been studied in detail, and is one of the best understood examples of how gene expression is regulated on the molecular level. The beta-galactosidase gene is controlled by segments of DNA adjacent to the gene. These regulatory DNA sequences (the general term used in Claim 1) include the operator and promoter sequences (specified in Claim 2).⁹ The researchers constructed a plasmid containing the beta-galactosidase gene with its operator and promoter. This gene (with its regulatory sequences) was removed from the chromosome of *E. coli* where it is normally found and was transplanted to a plasmid that could be conveniently manipulated.

25 Restriction endonucleases are useful tools in genetic engineering. These enzymes cut strands of DNA, but only at places where a specific sequence of nucleotides is present. For example, one restriction endonuclease, called EcoRI, cuts DNA only at sites where the nucleotide sequence is [...-G-A-A-T-T-C-...]. With restriction enzymes the genetic engineer can cut a strand of DNA at very specific sites into just a few pieces. With the help of "repair" enzymes, other pieces of DNA can be spliced onto the cut ends. The investigators found that the plasmid which they had constructed contained only two sequences that were cut by EcoRI. They were able to eliminate one of these sites that was unwanted. They were then left with a plasmid containing the beta-galactosidase gene with its regulatory sequences, and a single EcoRI site that was within the beta-galactosidase gene and close to its stop codon. They named this plasmid that they

« up ad constructed pBGP120.

26 The next step was to cut the plasmid open at its EcoRI site and insert a heterologous gene from another organism. The particular heterologous gene they chose to splice in was a segment of DNA from a frog that coded for ribosomal RNA. The frog gene was chosen as a test gene for reasons of convenience and availability. The new plasmid created by inserting the frog gene was similar to pBGP120, but its beta-galactosidase gene was incomplete. Some codons including the stop codon were missing from its end, which instead continued on with the sequence of the frog ribosomal RNA gene. The investigators named this new plasmid pBGP123. They inserted this plasmid back into E. coli and grew sufficient quantities for study. They then fed the E. coli with lactose. As they had intended, the lactose turned on transcription of the beta-galactosidase gene in the plasmid. RNA polymerase moved along the plasmid producing a strange new kind of RNA: Each long strand of RNA first contained codons for the messenger RNA for beta-galactosidase and then continued without interruption with the codons for the frog ribosomal RNA. Thus, there was readthrough transcription in which the RNA polymerase first transcribed the indigenous (beta-galactosidase) gene and then "read through," i.e., continued into and through the adjacent heterologous (frog ribosomal RNA) gene. Although the RNA produced was a hybrid, it nevertheless contained a nucleotide sequence dictated by DNA from a frog. The researchers had achieved the first controlled transcription of an animal gene inside a bacterium.

27 The researchers had used a gene coding for a ribosomal RNA as their heterologous test gene. Ribosomal RNA is not normally translated into protein. Nevertheless, they were obviously interested in using their approach to make heterologous proteins in bacteria. They therefore examined the beta-galactosidase made by their transformed bacteria. Patrick O'Farrell, who was not a coauthor of the Polisky paper but was to become a coinventor in the patent application, joined as a collaborator. They found that beta-galactosidase from the transformed bacteria had a higher molecular weight than was normal. They concluded that the bacteria must have used their strange new hybrid RNA like any other messenger RNA and translated it into protein. When the machinery of protein synthesis reached the premature end of the sequence coding for beta-galactosidase it continued right on, three nucleotides at a time, adding whatever amino acid was coded for by those nucleotides, until a triplet was reached with the sequence of a stop codon. The resulting polypeptide chains had more amino acids than normal beta-galactosidase, and thus a higher molecular weight. The researchers published their preliminary results in the Polisky article. They wrote:

28 [I]f the normal translational stop signals for [beta]-galactosidase are missing in pBGP120, in-phase translational readthrough into adjacent inserted sequences might occur, resulting in a significant increase in the size of the [beta]-galactosidase polypeptide subunit. In fact, we have recently observed that induced cultures of pBGP123 contain elevated levels of [beta]-galactosidase of higher subunit molecular weight than wild-type enzyme (P. O'Farrell, unpublished experiments). We believe this increase results from translation of Xenopus [frog] RNA sequences covalently linked to [messenger] RNA for [beta]-galactosidase, resulting in a fused polypeptide.

29 Polisky at 3904.

30 Since ribosomal RNA is never translated in normal cells, the polypeptide chain produced by translating that chain was not a naturally occurring, identified protein. The authors of the Polisky paper explicitly pointed out that if one were to insert a heterologous gene coding for a protein into their plasmid, it should produce a "fused protein" consisting of a polypeptide made of beta-galactosidase plus the protein coded

« up or by the inserted gene, joined by a peptide bond into a single continuous polypeptide chain:

31 It would be interesting to examine the expression of a normally translated eukaryotic sequence in pBGP120. If an inserted sequence contains a ribosome binding site that can be utilized in bacteria, production of high levels of a readthrough transcript might allow for extensive translation of a functional eukaryotic polypeptide. In the absence of an independent ribosome binding site, the eukaryotic sequence would be translated to yield a peptide covalently linked to [beta]-galactosidase. The extent of readthrough translation under lac control will depend on the number of translatable codons between the EcoRI site and the first in-phase nonsense [i.e., stop] codon in the inserted sequence.

32 Id.

III. The Claimed Invention

33 Referring back to Claims 1 through 3, it can be seen that virtually everything in the claims was present in the prior art Polisky article. The main difference is that in Polisky the heterologous gene was a gene for ribosomal RNA while the claimed invention substitutes a gene coding for a predetermined protein. Ribosomal RNA gene is not normally translated into protein, so expression of the heterologous gene was studied mainly in terms of transcription into RNA. Nevertheless, Polisky mentioned preliminary evidence that the transcript of the ribosomal RNA gene was translated into protein. Polisky further predicted that if a gene that codes for a protein were to be substituted for the ribosomal RNA gene, "a readthrough transcript might allow for extensive translation of a functional eukaryotic polypeptide." Thus, the prior art explicitly suggested the substitution that is the difference between the claimed invention and the prior art, and presented preliminary evidence suggesting that the method could be used to make proteins.

34 Appellants reduced their invention to practice some time in 1976 and reported their results in a paper that was published in 1978.¹⁰ During 1977 they communicated their results to another group of researchers who used the readthrough translation approach to achieve the first synthesis of a human protein in bacteria.¹¹ Appellants filed an application to patent their invention on August 9, 1978, of which the application on appeal is a division.

IV. The Obviousness Rejection

35 The application was rejected under 35 U.S.C. Sec. 103. The position of the examiner and the Board is, simply, that so much of the appellant's method was revealed in the Polisky reference that making a protein by substituting its gene for the ribosomal RNA gene in Polisky (as suggested by Polisky) would have been obvious to one of ordinary skill in the art at the time that the invention was made.

36 The claims specify that the heterologous gene should be inserted into the plasmid in the same orientation and with the same reading frame as the preceding portion of the indigenous gene. In view of this limitation, the Sec. 103 rejection was based either on Polisky alone (supplemented by the fact that the importance of orientation and reading frame was well known in the prior art) or in combination with the Bahl reference which describes a general method for inserting a piece of chemically synthesized DNA into a plasmid. Bahl teaches that this technique could be used to shift the sequence of DNA inserted into a plasmid into the proper reading frame.

37 Appellants argue that at the time the Polisky article was published, there was

« up Significant unpredictability in the field of molecular biology so that the Polisky article would not have rendered the claimed method obvious to one of ordinary skill in the art. Even though there was speculation in the article that genes coding for proteins could be substituted for the ribosomal RNA gene and would be expressed as readthrough translation into the protein, this had never been done. Appellants say that it was not yet certain whether a heterologous protein could actually be produced in bacteria, and if it could, whether additional mechanisms or methods would be required. They contend that without such certainty the predictions in the Polisky paper, which hindsight now shows to have been correct, were merely invitations to those skilled in the art to try to make the claimed invention. They argue that the rejection amounts to the application of a standard of "obvious to try" to the field of molecular biology, a standard which this court and its predecessors have repeatedly rejected as improper grounds for a Sec. 103 rejection. E.g., In re Fine, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1599 (Fed.Cir.1988); In re Geiger, 815 F.2d 686, 688, 2 USPQ2d 1276, 1278 (Fed.Cir.1987); In re Merck & Co., Inc., 800 F.2d 1091, 1097, 231 USPQ 375, 379 (Fed.Cir.1986); In re Antonie, 559 F.2d 618, 620, 195 USPQ 6, 8 (CCPA 1977).

38 Obviousness under Sec. 103 is a question of law. Panduit Corp. v. Dennison Mfg. Co., 810 F.2d 1561, 1568, 1 USPQ2d 1593, 1597 (Fed.Cir.), cert. denied, --- U.S. ----, 107 S.Ct. 2187, 95 L.Ed.2d 843 (1987). An analysis of obviousness must be based on several factual inquiries: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art at the time the invention was made; and (4) objective evidence of nonobviousness, if any. Graham v. John Deere Co., 383 U.S. 1, 17-18, 86 S.Ct. 684, 693-94, 15 L.Ed.2d 545, 556-57, 148 USPQ 459, 467 (1966). See, e.g., Custom Accessories, Inc. v. Jeffrey-Allan Indus., 807 F.2d 955, 958, 1 USPQ2d 1196, 1197 (Fed.Cir.1986). The scope and content of the prior art and the differences between the prior art and the claimed invention have been examined in sections II and III, *supra*. Appellants say that in 1976 those of ordinary skill in the arts of molecular biology and recombinant DNA technology were research scientists who had "extraordinary skill in relevant arts" and "were among the brightest biologists in the world." Objective evidence of nonobviousness was not argued.

39 With the statutory factors as expounded by Graham in mind and considering all of the evidence, this court must determine the correctness of the board's legal determination that the claimed invention as a whole would have been obvious to a person having ordinary skill in the art at the time the invention was made. We agree with the board that appellants' claimed invention would have been obvious in light of the Polisky reference alone or in combination with Bahl within the meaning of Sec. 103. Polisky contained detailed enabling methodology for practicing the claimed invention, a suggestion to modify the prior art to practice the claimed invention, and evidence suggesting that it would be successful.

40 Appellants argue that after the publication of Polisky, successful synthesis of protein was still uncertain. They belittle the predictive value of the observation that expression of the transcribed RNA in Polisky produced beta-galactosidase with a greater than normal molecular weight, arguing that since ribosomal RNA is not normally translated, the polypeptide chains that were added to the end of the beta-galactosidase were "junk" or "nonsense" proteins. This characterization ignores the clear implications of the reported observations. The Polisky study directly proved that a readthrough transcript messenger RNA had been produced. The preliminary observation showed that this messenger RNA was read and used for successful translation. It was well known in the art that ribosomal RNA was made of the same nucleotides as messenger RNA, that any sequence of nucleotides could be read in groups of three as codons, and that reading these codons should specify a polypeptide chain that would elongate until a stop codon was

« up ncountered. The preliminary observations thus showed that codons beyond the end of the beta-galactosidase gene were being translated into peptide chains. This would reasonably suggest to one skilled in the art that if the codons inserted beyond the end of the beta-galactosidase gene coded for a "predetermined protein," that protein would be produced. In other words, it would have been obvious and reasonable to conclude from the observation reported in Polisky that since nonsense RNA produced nonsense polypeptides, if meaningful RNA was inserted instead of ribosomal RNA, useful protein would be the result. The relative shortness of the added chains is also not a source of uncertainty, since one skilled in the art would have known that a random sequence of nucleotides would produce a stop codon before the chain got too long.¹²

41 Appellants complain that since predetermined proteins had not yet been produced in transformed bacteria, there was uncertainty as to whether this could be done, and that the rejection is thus founded on an impermissible "obvious to try" standard. It is true that this court and its predecessors have repeatedly emphasized that "obvious to try" is not the standard under Sec. 103. However, the meaning of this maxim is sometimes lost. Any invention that would in fact have been obvious under Sec. 103 would also have been, in a sense, obvious to try. The question is: when is an invention that was obvious to try nevertheless nonobvious?

42 The admonition that "obvious to try" is not the standard under Sec. 103 has been directed mainly at two kinds of error. In some cases, what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. E.g., *In re Geiger*, 815 F.2d at 688, 2 USPQ2d at 1278; *Novo Industri A/S v. Travenol Laboratories, Inc.*, 677 F.2d 1202, 1208, 215 USPQ 412, 417 (7th Cir.1982); *In re Yates*, 663 F.2d 1054, 1057, 211 USPQ 1149, 1151 (CCPA 1981); *In re Antonie*, 559 F.2d at 621, 195 USPQ at 8-9. In others, what was "obvious to try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it. *In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1532 (Fed.Cir.1988); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1380, 231 USPQ 81, 90-91 (Fed.Cir.1986), cert. denied, --- U.S. ----, 107 S.Ct. 1606, 94 L.Ed.2d 792 (1987); *In re Tomlinson*, 363 F.2d 928, 931, 150 USPQ 623, 626 (CCPA 1966). Neither of these situations applies here.

43 Obviousness does not require absolute predictability of success. Indeed, for many inventions that seem quite obvious, there is no absolute predictability of success until the invention is reduced to practice. There is always at least a possibility of unexpected results, that would then provide an objective basis for showing that the invention, although apparently obvious, was in law nonobvious. *In re Merck & Co.*, 800 F.2d at 1098, 231 USPQ at 380; *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1461, 221 USPQ 481, 488 (Fed.Cir.1984); *In re Papesch*, 315 F.2d 381, 386-87, 137 USPQ 43, 47-48 (CCPA 1963). For obviousness under Sec. 103, all that is required is a reasonable expectation of success. *In re Longi*, 759 F.2d 887, 897, 225 USPQ 645, 651-52 (Fed.Cir.1985); *In re Clinton*, 527 F.2d 1226, 1228, 188 USPQ 365, 367 (CCPA 1976). The information in the Polisky reference, when combined with the Bahl reference provided such a reasonable expectation of success.

44 Appellants published their pioneering studies of the expression of frog ribosomal RNA genes in bacteria more than a year before they applied for a patent. After providing virtually all of their method to the public without applying for a patent within a year, they foreclosed themselves from obtaining a patent on a method that would have been obvious

« up 'om their publication to those of ordinary skill in the art, with or without the disclosures
f other prior art. The decision of the board is

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AFFIRMED.

¹ Basic background information about molecular biology and genetic engineering, can be found in Alberts, Bray, Lewis, Raff, Roberts & Watson, *The Molecular Biology of the Cell*, 1-253, 385-481 (1983) [hereinafter *The Cell*]; Watson, Hopkins, Roberts, Steitz & Weiner, *The Molecular Biology of the Gene*, Vol. 1 (4th ed., 1987) 3-502 [hereinafter *The Gene*]. These standard textbooks were used to supplement the information in the glossary supplied by appellants. The description here is necessarily simplified and omits important facts and concepts that are not necessary for the analysis of this case

² There are twenty amino acids: alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine, aspartic acid, glutamic acid, lysine, arginine, and histidine

³ Proteins are often loosely called peptides, but technically proteins are only the larger peptides with chains of at least 50 amino acids, and more typically hundreds of amino acids. Some proteins consist of several polypeptide chains bound together covalently or noncovalently. The term "peptide" is broader than "protein" and also includes small chains of amino acids linked by peptide bonds, some as small as two amino acids. Certain small peptides have commercial or medical significance

⁴ Polypeptide chains fold up into complex 3-dimensional shapes. It is the shape that actually determines many chemical properties of the protein. However, the configuration of a protein molecule is determined by its amino acid sequence. *The Cell* at 111-12; *The Gene* at 50-54

⁵ The sugar in DNA is deoxyribose, while the sugar in RNA, infra, is ribose. The sugar and phosphate groups are linked covalently to those of adjacent nucleotides to form the backbone of the long unbranched DNA molecule. The bases project from the chain, and serve as the "alphabet" of the genetic code

DNA molecules actually consist of two chains tightly entwined as a double helix. The chains are not identical but instead are complementary: each A on one chain is paired with a T on the other chain, and each C has a corresponding G. The chains are held together by noncovalent bonds between these complementary bases. This double helical structure plays an essential role in the replication of DNA and the transmission of genetic information. See generally *The Cell* at 98-106; *The Gene* at 65-79. However, the information of only one strand is used for directing protein synthesis, and it is not necessary to discuss the implication of the double-stranded structure of DNA here. RNA molecules, infra, are single stranded.

⁶ Chromosomes also contain regions of DNA that are not part of genes, i.e., do not code for the sequence of amino acids in proteins. These include sections of DNA adjacent to genes that are involved in the control of transcription, infra, and regions of unknown function

⁷ See *The Cell* at 185-194; *The Gene* at 208-10

⁸ Polisky, Bishop & Gelfand, A plasmid cloning vehicle allowing regulated expression of eukaryotic DNA in bacteria, 73 Proc.Nat'l Acad.Sci. USA 3900 (1976)

⁹ The promoter is a sequence of nucleotides where the enzyme that synthesizes RNA, RNA polymerase, attaches to the DNA to start the transcription of the beta-galactosidase gene. The operator is an overlapping DNA sequence that binds a small protein present in the cell, the lactose repressor protein. The lactose repressor protein binds to the operator and physically blocks the RNA polymerase from properly attaching to the promoter so that transcription cannot proceed. Lactose molecules interact with the lactose repressor protein and cause it to change its shape; after this change in shape it moves out of the way and no longer prevents the RNA polymerase from binding to the promoter. Messenger RNA coding for beta-galactosidase can then be

« up transcribed. See generally *The Cell* at 438-39; *The Gene* at 474-80

¹⁰ O'Farrell, Polisky & Gelfand, Regulated expression by readthrough translation from a plasmid-encoded beta-galactosidase, 134 J. Bacteriol. 645 (1978). The heterologous genes expressed in these studies were not predetermined, but were instead unidentified genes of unknown origin. The authors speculated that they were probably genes from *E. coli* that were contaminants in the source of beta-galactosidase genes. *Id.* at 648

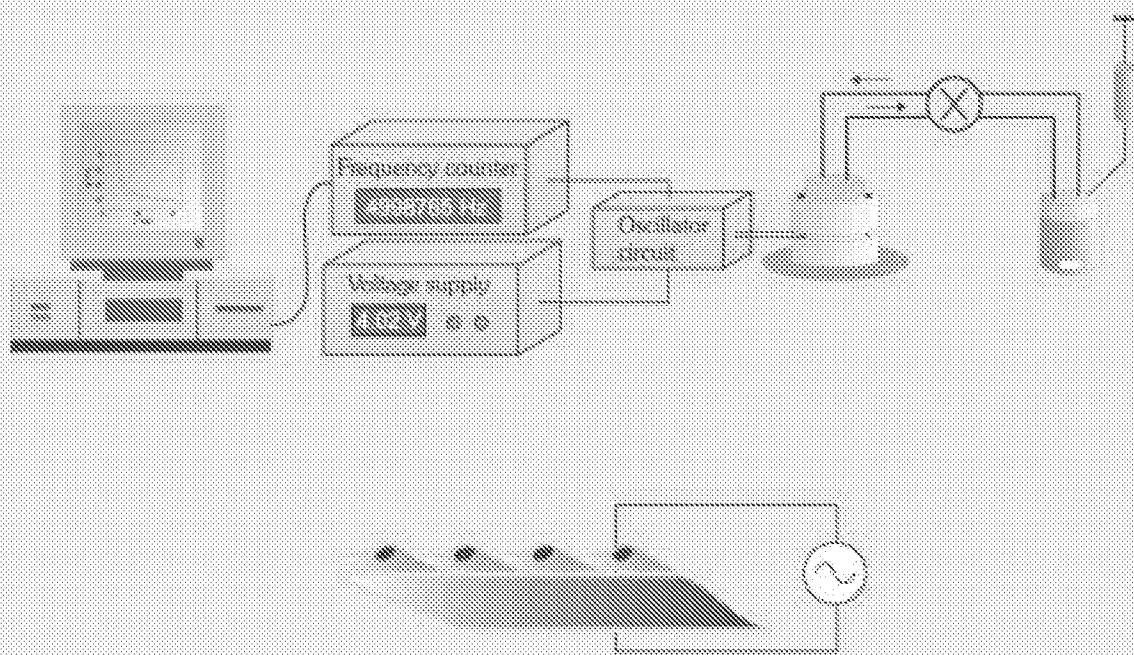
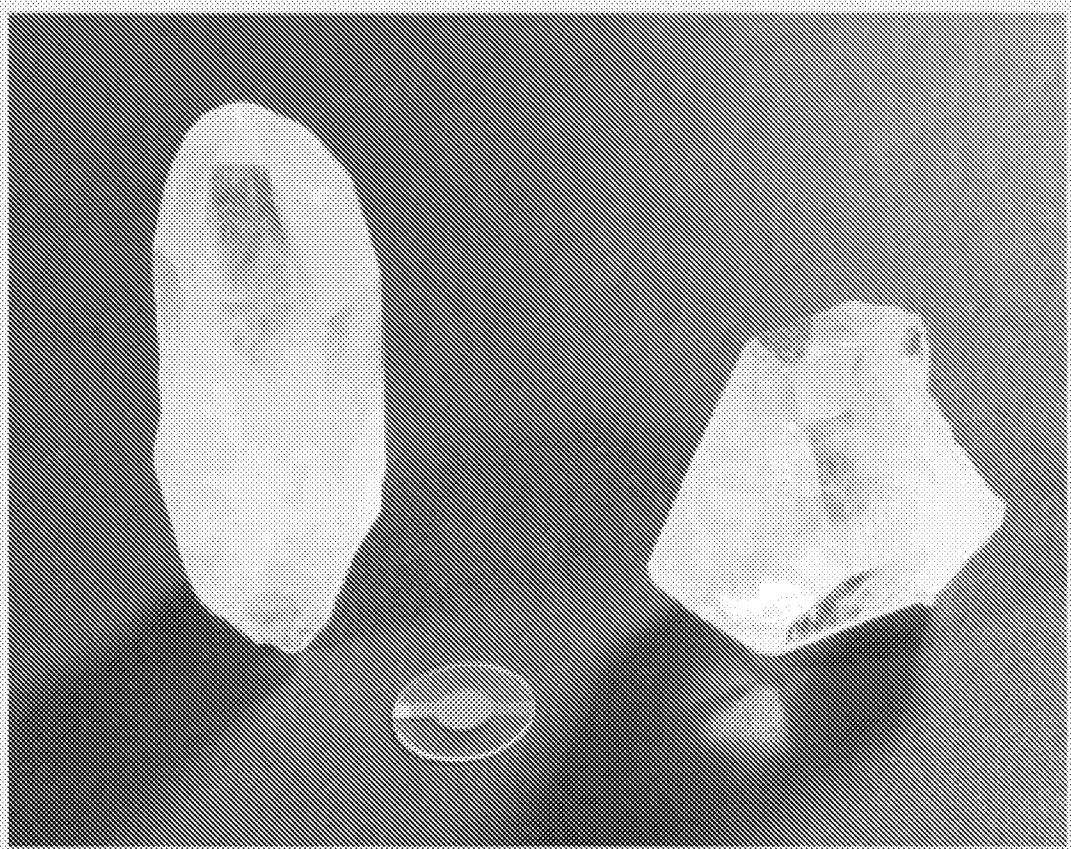
¹¹ Itakura, Hirose, Crea, Riggs, Heynecker, Bolivar & Boyer, Expression in *Escherichia coli* of a chemically synthesized gene for the hormone somatostatin, 198 Science 1056 (1977). A pioneering accomplishment of the Itakura group is that the gene was not from a human source, but instead was entirely synthesized in the laboratory using chemical methods. It is not clear whether the appellants communicated only the results reported in the Polisky publication or whether they communicated the complete claimed invention

¹² The patent application indicates that chains as long as 60 amino acids were added, which is hardly a trivial length of polypeptide



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THE QUARZ-CRYSTAL MICROBALANCE IN LIFE SCIENCE



Piezoelectric Mass-Sensing Devices as Biosensors—An Alternative to Optical Biosensors?

Andreas Janshoff, Hans-Joachim Galla, and Claudia Steinem*

Dedicated to Professor Erich Sackmann on the occasion of his 65th birthday

In the early days of electronic communication—as a result of the limited number of quartz resonators available—frequency adjustment was accomplished by a pencil mark depositing a foreign mass layer on the crystal. In 1959, Sauerbrey showed that the shift in resonance frequency of thickness-shear-mode resonators is proportional to the deposited mass. This was the starting point for the development of a new generation of piezoelectric mass-sensitive devices. However, it was the development of new powerful oscillator circuits that were capable of operating thickness shear mode resonators in fluids that enabled this technique to be introduced into bioanalytic applications. In the last decade adsorption of biomolecules on functionalized

surfaces turned into one of the paramount applications of piezoelectric transducers. These applications include the study of the interaction of DNA and RNA with complementary strands, specific recognition of protein ligands by immobilized receptors, the detection of virus capsids, bacteria, mammalian cells, and last but not least the development of complete immunosensors. Piezoelectric transducers allow a label-free detection of molecules; they are more than mere mass sensors since the sensor response is also influenced by interfacial phenomena, viscoelastic properties of the adhered biomaterial, surface charges of adsorbed molecules, and surface roughness. These new insights have recently been used to investigate the adhesion of cells, lip-

osomes, and proteins onto surfaces, thus allowing the determination of the morphological changes of cells as a response to pharmacological substances and changes in the water content of biopolymers without employing labor-intensive techniques. However, the future will show whether the quartz-crystal microbalance will assert itself against established label-free sensor devices such as surface plasmon resonance spectroscopy and interferometry.

Keywords: analytical methods • biosensors • molecular recognition • quartz-crystal microbalance • surface chemistry

1. Introduction

As a consequence of their extraordinary properties quartz resonators can be found in all kinds of electronic devices, such as watches and computers to give an accurate time base, and as signal generators or reference systems in electronic devices. Quartz resonators did not become of interest commercially until immediately prior to World War II when there was an enormous requirement for communication devices. More than 30 million resonators were necessary to cover the demand. Quartz crystals attained significance as an analytical device after the discovery that there is linear relationship

between deposited mass and the frequency response,^[1, 2] as demonstrated by Sauerbrey in 1959.^[3] He showed that this proportionality only holds if the ideal layer of foreign mass is strongly coupled to the resonator. This is the reason for calling such a device a “quartz-crystal microbalance” (QCM). The mass sensitivity of a 5 MHz quartz crystal is approximately $0.057 \text{ Hz cm}^2 \text{ ng}^{-1}$, which is approximately 100 times higher than that of an electronic fine-balance with a sensitivity of $0.1 \mu\text{g}$. In the 1960s and 1970s the QCM technique gained importance as devices for monitoring thicknesses in vacuum and air, and is still used in today’s laboratories to determine the thicknesses of layers. The lack of suitable oscillator circuits that enabled the shear-wave resonator to be operated in fluids prevented, however, the extension of this technique to bioanalytical areas. In 1982, Nomura and Okuhara^[4] were the first to report on a circuitry capable of breaking this barrier, thus giving the starting point for the development of a new class of bioanalytic tools.

[*] Dr. C. Steinem, Dr. A. Janshoff, Prof. Dr. H.-J. Galla
Institut für Biochemie
Westfälische Wilhelms-Universität
Wilhelm-Klemm-Strasse 2, 48149 Münster (Germany)
Fax: (+49) 251-83-33-206
E-mail: steinec@uni-muenster.de

Nowadays quartz resonators can be regarded as typical mechanical transducers for chemical and biological sensor devices that transform the mass or thickness of a foreign layer (physical quantity) of an analyte into an electrical signal. A mass adsorbed on a shear-wave resonator experiences an acceleration of more than 10^6 g . The amplitude of vibration is usually 10–20 nm in air and is reduced in water to a mere 1–2 nm.^[5]

This article is intended to show that specific interactions between biomolecules can be quantified in terms of thermodynamic and kinetic parameters by means of QCM. Furthermore, the objective is to put emphasis on the fact that the QCM is more than just a mass sensor. A new application of this classical technique is the determination of viscoelastic properties of cellular systems. The strength of piezoelectric transducers is that they can couple mechanical and electrical variables, which allows the formulation of equivalent circuits that describe the mechanical properties of biopolymers and

complex multilayers, such as confluent cell monolayers. Thus, it is possible to determine molecular recognition events apart from interfacial phenomena, surface energy, viscoelasticity, roughness, surface charge density, and the water content of biomolecules.

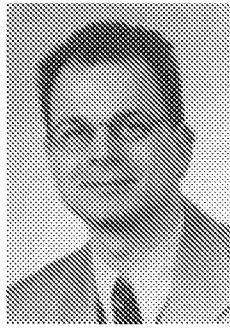
2. Basic Piezoelectric Resonators

2.1. Acoustic Waves in Solids

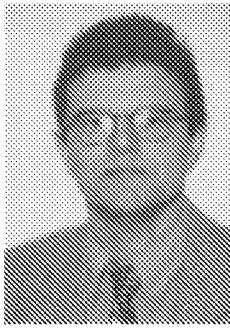
Acoustic waves cover a frequency range of 14 orders of magnitude—from 10^{-2} Hz (seismic waves) and extending to 10^{12} Hz (thermoelastic excited phonons; Figure 1). The acoustic resonators such as those mentioned in this article oscillate in a narrow frequency range of 10^6 – 10^9 Hz .

A brief introduction to acoustic wave theory and in particular piezoelectric excited vibrations is given and then

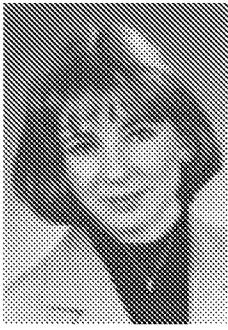
Andreas Janshoff was born in 1966. He attended the Westfälische Wilhelms-University (WWU) Münster, where he received a B.S. in biology and chemistry. He received his M.S. in 1994 and his Ph.D. in 1997 for studies on the biofunctionalization of gold surfaces and its applications in bioanalytics, which he carried out at the Institute for Biochemistry at the university of Münster. As a postdoctoral fellow, he attended the group of M. Reza Ghadiri at the Scripps Research Institute, La Jolla (CA), USA for 18 months. During this time he worked on the imaging of lipid membranes by scanning force microscopy. In 1999 he returned to Germany, joining the group of Harald Fuchs at the WWU Münster to start his habilitation in close collaboration with the Institute for Biochemistry (WWU). His research fields include the development of new sensor systems based on micropatterned surfaces and the application of acoustic resonators as a tool to investigate biological relevant questions.



A. Janshoff



H.-J. Galla



C. Steinem

Hans-Joachim Galla, born in 1948, attended the university of Göttingen. After receiving his B.S. in chemistry in 1973, he received his Ph.D. in 1975 in Göttingen under the tutelage of Erich Sackmann for work on phase-separation phenomena and lipid–protein interactions carried out at the Max-Planck Institute for Biophysical Chemistry. In 1976 he changed to the Department of Biophysics at the university of Ulm as a research associate, where he finished his habilitation in 1979. At the same time (1979/1980) he was a research associate at Stanford university before he returned to the university of Ulm as a Heisenberg fellow. In 1983 he became professor of biophysical chemistry at the Institute for Biochemistry of the technical university of Darmstadt. In 1990 he took a professorship of biochemistry at the university of Münster. His main research fields include the differentiation of cerebral endothelia of the blood–brain barrier, monomolecular lipid–peptide layers as model systems for the alveolar lung surfactant, and biofunctionalization of solid-supported lipid membranes for applications in bioanalytics.

Claudia Steinem, born in 1967, attended the Westfälische Wilhelms-University in Münster, where she received a B.S. in biology and chemistry and a M.S. in chemistry. In 1997 she received her Ph.D. under the tutelage of Hans-Joachim Galla on studying the ion transport in solid-supported lipid bilayers. After a postdoctoral stay at the Scripps Research Institute in La Jolla, CA (USA) for 18 months in the group of M. Reza Ghadiri she returned as Lise-Meitner fellow at the Institute for Biochemistry of the Westfälische Wilhelms-University in 1999. She is currently organizing her own research group in the course of her habilitation. Her main research interests include the study of the interaction of artificial peptides, annexins, and proteins of the signal transduction pathway with lipids, and the development of new lipid membrane systems based on porous surfaces.

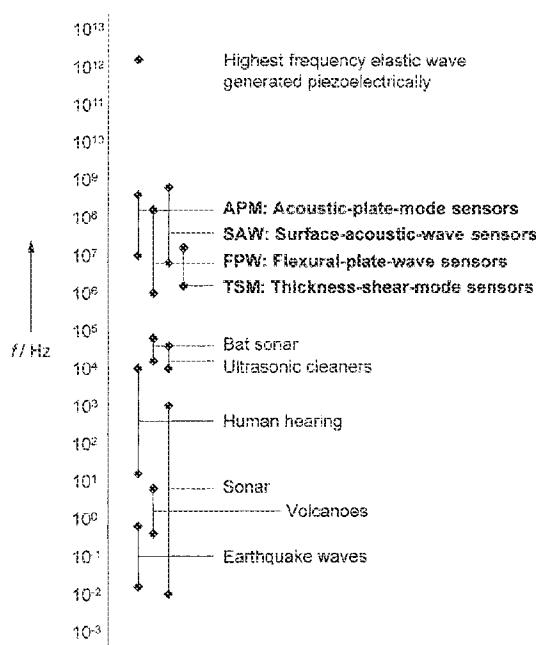


Figure 1. The spectrum of acoustic waves covers roughly 14 orders of magnitude. The frequency range of the four resonators which are described in this article range in operation between 1 and 1000 MHz.^[7]

this is followed by a more detailed description of different types of piezoelectric transducers.

The application of a periodic perturbation (stress) to a solid results in elastic deformations (strain), which travel as waves through the solid. The type of wave—transversal or longitudinal—and the phase velocity both depend on the crystal structure. The wave equation that describes acoustic waves in solids [Eq. (1)] can be derived using the equation of motion, the definition of strain, and the constitutive equations.^[6]

$$\nabla \cdot \mathbf{c} : \nabla_s \mathbf{u} = \rho \frac{\partial^2 \mathbf{u}}{\partial t^2} \quad (1)$$

\mathbf{u} is the displacement of the particle, ρ the density of the material, and t denotes time. The matrix \mathbf{c} describes the elasticity moduli of the solid. Table 1 summarizes the relevant expressions for one and three dimensions.

Table 1. The dynamic equations of motion for one and three dimensions. \mathbf{c} is the tensor of elasticity. Stress \mathbf{T} and strain \mathbf{S} are symmetric tensors with nine elements of which six are independent because of symmetry. \mathbf{u} is the particle displacement, z indicates the direction where strain occurs, and ρ is the density of the material.^[6]

	One-dimensional system	Three-dimensional system
Newton's law	$\frac{\partial T}{\partial z} = \rho \frac{\partial^2 u}{\partial t^2}$	$\nabla \cdot \mathbf{T} = \rho \frac{\partial^2 \mathbf{u}}{\partial t^2}$
definition of stress	$S = \frac{\partial u}{\partial z}$	$\mathbf{S} = \nabla_s \mathbf{u}$
constitutive relations		
Hooke's law	$T = CS$	$\mathbf{T} = \mathbf{c} : \mathbf{S}$
particle velocity	$v = \frac{\partial u}{\partial t}$	$v = \frac{\partial \mathbf{u}}{\partial t}$
mechanical impedance	$Z = \sqrt{\rho C}$	
phase rate	$v_p = \sqrt{C/\rho}$	

2.2. Piezoelectric Excited Acoustic Waves in Solids

Piezoelectricity as first reported 1880 by the Curie brothers describes the generation of electrical charges on the surface of a solid caused by pulling, pressure, or torsion. In contrast, the occurrence of a mechanical deformation arising from an external electric field is called the converse piezoelectric effect. Figure 2 shows schematically the general relationships between mechanical and electrical variables.

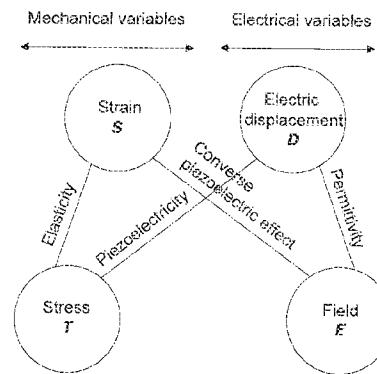


Figure 2. Relationship between mechanical and electrical variables. The direct piezoelectric effect is the production of electric displacement by applying a mechanical stress. The converse piezoelectric effect results in a strain in the crystal when an electrical field is applied. The relation between stress and strain is determined by the elasticity of the solid.^[7]

Prerequisite for the occurrence of piezoelectricity in crystals is an inversion center. In total 21 point groups fulfill this requirement but only 20 classes have nonzero piezoelectric constants. Although a large number of crystals show piezoelectricity only quartz provides the unique combination of mechanical, electrical, chemical, and thermal properties, which has led to its commercial significance. Equation 1 needs to be expanded by the term $e \nabla^2 \Phi$ in order to introduce piezoelectricity in the general wave equation, in which Φ is the electrical potential and e the piezomodul composed of piezoelectric constants as matrix elements.^[7] The additional expression is responsible for the electrical excitation of elastic waves in a piezoelectric solid. The resulting motion causes a change in Φ , which can in turn be detected electrically. Since the gradient of the displacement current is zero, that is, no free charge density occurs, integration of the extended Equation (1) results in a comprehensive expression that describes acoustic waves in a piezoelectric medium. The following section deals with its physical consequences.

2.3. Piezoelectric Resonators

The main emphasis of this article is placed on bulk-acoustic-wave (BAW) or thickness-shear-mode (TSM) resonators, which are also known as quartz-crystal microbalances (QCMs). Although TSM resonators are considerably less sensitive than flexural-plate-wave (FPW) or surface-acoustic-wave (SAW) sensors they are widely used as a result of their robust nature, availability, and affordable electronics. Figure 3 depicts schematically various resonator types.

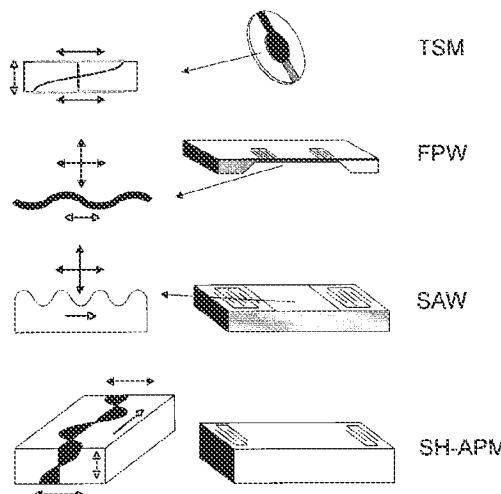


Figure 3. Schematic sketches of the four common types of acoustic resonators and their wave propagation modes. The particle displacement is indicated by a black arrow, and the direction of the wave propagation by an open arrow. TSM: thickness-shear-mode resonator, also known as the quartz-crystal microbalance technique; FPW: flexural-plate-wave resonator; SAW: surface-acoustic-wave resonator (two port delay line) and SH-APM: shear-horizontal-acoustic-plate-mode resonator.

2.3.1. Thickness-Shear-Mode (TSM) Resonators (Quartz-Crystal Microbalances)

Depending on the cut-angle a large number of different resonator types such as thickness-shear-mode, plate, and flexural resonators can be obtained from a mother crystal (Figure 4 A) with eigenfrequencies ranging from 5×10^2 – 3×10^8 Hz. Generally, AT-cut crystals are used for QCM purposes, being cut with an angle of 35.25° to the z -axis (Figure 4 B). AT-cut quartz crystals exhibit a high frequency stability of $\Delta f/f \approx 10^{-8}$, which makes them well-suited for many electronic devices.^[8, 9]

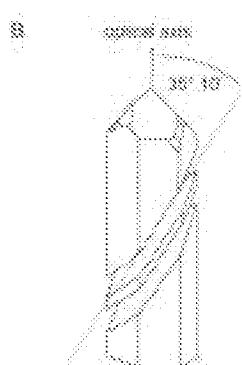
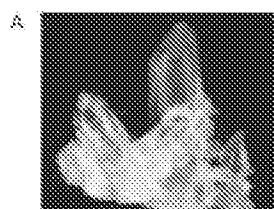


Figure 4. A) Photograph of several α -quartz crystals. B) AT-cut of a quartz crystal. A quartz plate is cut at an angle of $35^\circ 10'$ with respect to the optical axis. A deviation of only $5'$ from the angle leads to a temperature coefficient that is different from zero in the range of 0 – 50°C .

Since AT-cut quartz crystals have a temperature coefficient that is almost zero between 0 – 50°C , this particular cut is the most suitable one for QCM sensors.^[10] The following parameters are always related to AT-cut quartz.

A convenient way to describe acoustic waves traveling in a circular, lossless, and stress-free AT-cut crystal with thickness d_q is to express the wave equation in cylindrical coordinates (Figure 5). The propagation velocity of the

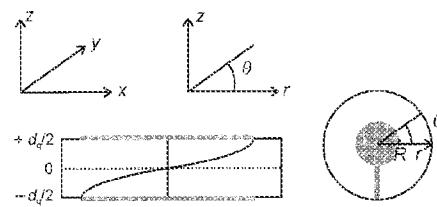


Figure 5. Definition of the coordinates of a circular-shaped quartz crystal. The gray areas depict the metal electrodes.

acoustic wave in a piezoelectric thickness-shear-mode resonator is $(\bar{c}_{66}/\rho_q)^{1/2}$ (ρ_q = density of the quartz).^[11] The boundary conditions require that the amplitude of the shear displacement is zero at the electrode edges, while it is at a maximum at $r=0$ and exhibits a node at $z=0$. θ follows periodic boundary conditions. The wave equation can be solved by a usual separation process.^[12] The solution yields important properties of the shear vibration. The eigenfrequencies are found in general from Equation (2).^[9]

$$f_{mnk} = \frac{v_p}{2\pi} \sqrt{\frac{n^2\pi^2}{d_q^2} + \frac{\lambda_{mk}^2}{R^2}} \approx \frac{v_p}{2\pi} \sqrt{\frac{n^2\pi^2}{d_q^2}} = \frac{v_p n}{2 d_q} = \frac{n K_R}{d_q} \quad (2)$$

From the solution of the wave equation, it is clear that only odd overtones n can be excited.^[13] The displacement at $r=0$ and $z=\pm d_q/2$ is at a maximum for the fundamental frequency, while the amplitude vanishes at the electrode edges where $r=R$. Thus, the transversal wave exhibits a node at $z=0$ and maximum amplitude at $z=\pm d_q/2$.^[14]

If the lateral dimensions of the quartz plate greatly exceeds the thickness of the crystal the problem can be treated one-dimensionally. The function $u_r(z, t)$ describes two transversal waves traveling in opposite directions, which thus form a standing wave within the crystal. Assuming stress-free surfaces ($\partial u/\partial z=0$), the shear vibration can be described as a simple cosine function. The use of constructive interference $d_q=n\lambda/2$ and $R \gg d_q$ simplifies Equation (2).^[15] K_R denotes the so-called frequency constant of AT-cut quartz with $K_R=1664 \text{ m s}^{-1}$.^[9] It can be deduced from Equation (2) that the resonance frequency of an AT-cut quartz increases with decreasing thickness of the crystal. For instance, a 5 MHz quartz exhibits a thickness of 0.33 mm, while a 30 MHz crystal is only 55 μm thick.

TSM resonators provide numerous material-specific parameters. In order to fully exploit the capability of acoustic resonators it is essential to understand the conversion between mechanical and electrical parameters since the last ones are those which are readily accessible to the user. Mechanical models can readily be transformed into equivalent electrical circuits which permit a complete description of the oscillation in the presence of an adsorbent. A general one-dimensional acoustic wideband model has been suggested by Mason^[16] (Figure 6 A) and provides a basis for the theoretical description of complex composite resonators as they occur in life science. In particular cases, such as low load, the Mason model can be easily transformed near resonance into an equivalent circuit with lumped elements—the so termed Butterworth–van-Dyke (BVD) circuit (Figure 6 B). A comprehensive treatment of this issue has been given by Rosenbaum.^[6]

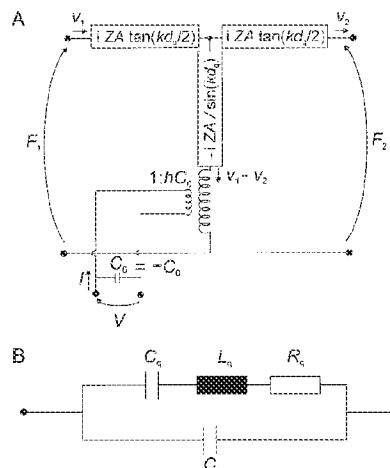


Figure 6. A) Equivalent circuit (wideband model) of the three-port Mason model of a finite thickness piezoelectric layer. The equivalent circuit consists of two mechanical ports and one electrical port. Z is the characteristic impedance of the quartz, k the complex wave number, A the area, and d_q the thickness of the resonator, hC_0 is the turns ratio of the transformer and is defined as $(e_{26}/e_{22})C_0$. B) Butterworth-van-Dyke-equivalent circuit. Near resonance the three-port Mason model can be transformed into an equivalent circuit composed of discrete impedance elements. The capacitance C_q represents the mechanical elasticity of the quartz, the inductance L_q the initial mass, and the resistance R_q the energy losses arising from viscous effects, internal friction, and damping induced by the crystal holder. The static capacitance C_0 determines the admittance away from resonance, while the motional components dominate near resonance.^[6]

The BVD circuit combines a parallel and series resonance circuit (motional branch). The motional branch consists of L_q , C_q , and R_q . The electrodes on both sides of the crystal plate provide an additional parallel capacitance C_0 , which gives rise to a parallel circuit. Table 2 summarizes the expressions for L_q , C_q , R_q , and C_0 as they occur at the fundamental vibration.

The quartz material between the two electrodes (Figure 5) serves as a typical dielectric material, thus the resonator behaves as a plate condenser at high frequencies. Figure 7 displays parameter curves of impedance spectra of a 5 MHz quartz crystal on varying L_q , C_q , R_q , and C_0 . The motional resistance R_q contains intrinsic viscosities of the quartz and is responsible for energy dissipation. The phase maximum is a measure of the damping of a quartz resonator, as pointed out by Martin et al.^[17]

If damping is negligible ($R_q \rightarrow 0$) the quartz resonator shows two resonance frequencies corresponding to a phase shift $\varphi = 0$ at minimum and maximum magnitudes of the impedance $|Z|$. The corresponding resonant frequencies are referred to as resonant frequency f_R and antiresonant frequency f_A .^[9, 18, 19] The separation between the frequencies rises with an increase in the electroacoustic coupling constant K . Four different resonant frequencies

Table 2. Parameters of the BVD-equivalent circuit and their relationship to the physical characteristic numbers of an AT-cut quartz together with the different resonance frequencies. A is the electrode area, η_q the viscosity, d_q the thickness of the quartz, ϵ_{22} the dielectric constant of the quartz material, and e_{26} the piezoelectric constant dependent on the cutting angle, which can be obtained from the matrix elements e_{11} and e_{14} of the piezoelectric polarization modulus e and the cutting angle. The material constants shown in this Table are only valid for AT-cut quartz.^[9]

Parameter	Expression
C_0	$\frac{\epsilon_{22}A}{d_q}$
C_q	$\frac{8Ae_{26}}{\pi^2 d_q \epsilon_{66}}$
L_q	$\frac{d_q^3 \rho_q}{8Ae_{26}^2}$
R_q	$\frac{d_q \eta_q \pi^2}{8Ae_{26}^2}$
f_s	$\frac{1}{2\pi} \sqrt{\frac{1}{L_q C_q}} \left(1 + \frac{C_0 R_q^2}{2L_q} \right)$
f_p	$\frac{1}{2\pi} \sqrt{\frac{1}{L_q C_q}} \left(1 + \frac{C_q}{2C_0} - \frac{C_0 R_q^2}{2L_q} \right)$
$f_{Z_{\min}}$	$\frac{1}{2\pi} \sqrt{\frac{1}{L_q C_q}} \left(1 - \frac{C_0 R_q^2}{2L_q} \right)$
$f_{Z_{\max}}$	$\frac{1}{2\pi} \sqrt{\frac{1}{L_q C_q}} \left(1 + \frac{C_q}{2C_0} + \frac{C_0 R_q^2}{2L_q} \right)$

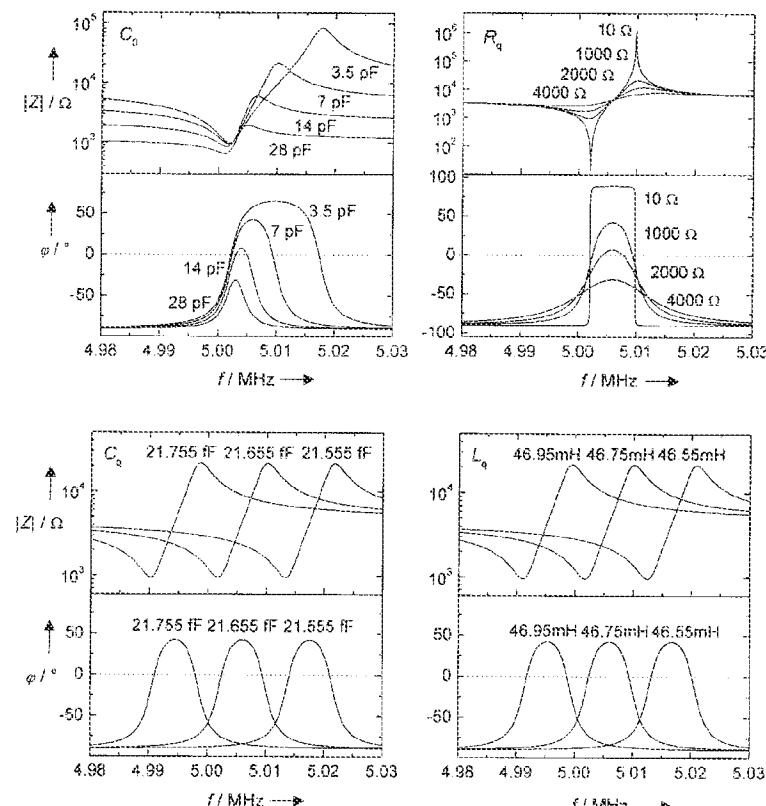


Figure 7. Simulated impedance spectra from the BVD-equivalent circuit with discrete variations of the impedance element values C_0 , C_q , L_q , and R_q .

are discernable if damping occurs ($R_q > 0$; Figure 8 A). f_R separates into $f_{Z\min}$, the frequency at minimal impedance ($|Z|$), and f_s , the frequency at zero phase φ at the low frequency branch. In turn f_A separates into f_p , the frequency at zero phase at the high frequency branch, and $f_{Z\max}$, the frequency at maximum impedance. The expressions for f_s , f_p ,

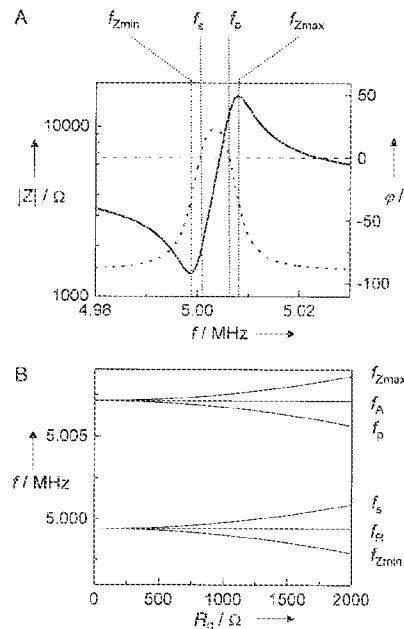


Figure 8. A) Simulated impedance spectrum from the BVD-equivalent circuit with marked resonance frequencies $f_{Z\min}$, f_s , f_p , and $f_{Z\max}$ for the case of $R_q > 0$. B) Dependence of the resonance frequencies $f_{Z\min}$, f_s , f_p , and $f_{Z\max}$ on the motional resistance R_q calculated according to the approximation of Bottom.^[9] Accurate calculations show that f_s and f_p converge at $R_q = 2280 \Omega$.

$f_{Z\min}$, and $f_{Z\max}$ assuming low R_q and their dependencies on the electrical parameters of the simple BVD equivalent circuit are given in Table 2.

The dependence of the resonance frequency on the damping resistance R_q are shown in Figure 8B. $f_{Z\max}$ and f_s rise with increasing damping, while $f_{Z\min}$ and f_p decrease. The resonant frequencies f_s and f_p converge at $\varphi_{\max} = 0$.

Active oscillation of shear resonators is restricted to $\varphi_{\max} > 0$. At larger resistances leading to $\varphi_{\max} \leq 0$ impedance analysis by a network analyzer is necessary to further study the behavior of the resonator.

2.3.1.1. Mass Loading

Sauerbrey has provided the first treatment of this issue.^[3] By analytically solving the one-dimensional equation of motion he showed that an ideal layer of foreign mass results in a frequency decrease Δf that is proportional to the deposited mass Δm if the resonator is operated in air or vacuum. If the density of the

mass layer is equal to that of the quartz crystal, Equation (3) applies.

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\epsilon_{66}\rho_q}} \Delta m = -S_f \Delta m \quad (3)$$

Equation (3) describes the frequency response of a resonator on deposition of thin, rigid, and uniform films. The integral mass sensitivity or Sauerbrey constant S_f depends on the square of the fundamental frequency [Eq. (3)], and increases proportionally to the overtone number.

The differential mass sensitivity, however, is maximum in the center of the quartz resonator and decreases towards the borders of the electrodes (Figure 9).

The concept of energy trapping explains the differential mass sensitivity observed.^[6] The conditions for resonance and hence the resonant frequencies of the quartz crystal in the electrode-free region are different from those at the electrodes as a result of the additional mass of the electrodes. Analogous to the total reflection of light in an optical waveguide, in which the incoming light cannot penetrate the optically more dense material, standing acoustic waves generated at the electrodes are confined to this region, and is referred to as energy trapping. The lateral components of the acoustic wave, which travel tangential to the surface of the crystal are almost completely reflected at the interface between the electrode and electrode-free region. Energy trapping suppresses spurious modes and the quality factor of the resonator increases.^[20] However, if the thickness of the electrodes is too thin (usually less than 500 Å) the resonant frequencies of regions with and without electrodes are very similar, so that the acoustic wave is not confined to the electrode-covered region, and as a consequence the quality factor Q of the resonator decreases. Very thick electrodes, however, cause a decrease in the Q -factor as a result of the

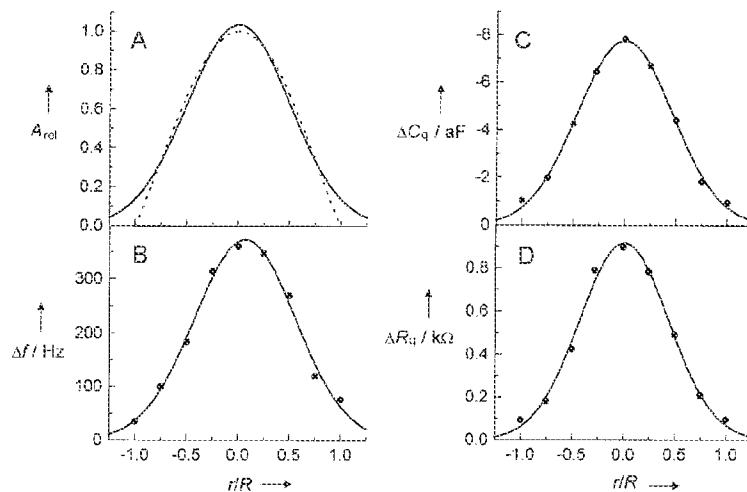


Figure 9. A) Radial distribution of the relative oscillation amplitude at the quartz surface according to a Bessel distribution (dotted line) and a Gaussian distribution (solid line) with $a = 2$. The Bessel function drops to zero at the electrode edges where $r/R = 1$, whereas the Gaussian function includes motion near the electrode edges. B) The increase in frequency of a 5 MHz AT-cut quartz while pressing a polyethylene tip on the quartz surface immersed in water. The change in resonance frequency is defined as $\Delta f = f(r) - f_0$. C) Corresponding capacitance change ΔC_q of a 5 MHz AT-cut quartz. D) Change in motional resistance ΔR_q . The solid lines are the results of the fitting parameters of a Gaussian distribution to the data.

presence of a dead dielectric, which is particularly pronounced by using gold electrodes and high frequency resonators.^[21] Electrodes made of aluminum instead of gold improve the situation because of the lower acoustic adsorption coefficient of aluminum. Further improvement can be achieved by using larger electrode areas.

The radial mass sensitivity s_f is proportional to the square of the radial displacement ($s_f(r) \propto |u(r)|^2$),^[22] which is described by a Bessel function J_0 of the first kind and zeroth order (Figure 9 A).^[12] The highest sensitivity is located at the center of the crystal at $r=0$ and vanishes at the electrode edges. However, Martin and Hager^[23] were the first to show that the amplitude of vibration is nonzero beyond the electrode edges ($r=R$) as a result of field fringing (inhomogeneous electric field, Figure 16), which is not considered by the energy-trapping concept. Field fringing is enhanced in an environment of higher permittivity such as water. The amplitude of the shear vibration depends on energy dissipation and therefore on the kind of load on the quartz. Liquid loading usually results in considerable damping and increased field fringing leading to a broadening of the curve and a decreased amplitude. The radial distribution of the shear amplitude can empirically be described by a Gaussian function [Eq. (4)].^[23]

$$u(r) = u_{\max} \exp\left(-a \frac{r^2}{R^2}\right) \quad (4)$$

u_{\max} denotes the maximum displacement at $r=0$ and a is the characteristic width of the distribution, with typical values of $a \approx 2$ for a plane-parallel 5 MHz quartz in water. Figure 9 A displays a comparison of the two distributions. The spatial distribution of the differential mass sensitivity can be experimentally probed by depositing small defined masses or attaching a sharp probe at certain locations of the crystal surface. The latter approach has been used to map the sensitivity distribution of a 5 MHz quartz resonator as shown in Figure 9 B–D. The maximum change of Δf , ΔR_q , and ΔC_q occurs in the center of the plate. The distribution is well described by an empiric Gaussian function. By including field fringing, Hillier and Ward^[12] provided a solution of the wave equation that shows proportionality between frequency shift and deposited mass only if the material is homogeneously distributed over the crystal. Employing electrolysis to calibrate the QCM, however, does not always result in the formation of homogeneous metal films but often leads to an increased amount of deposited material at the electrode edges, which thus results in a systematic underestimation of the integral mass sensitivity.

The Sauerbrey Equation does not apply for thick films, viscous liquids, elastic solids, and viscoelastic bodies. In order to describe these various types of loading, theoretical models are needed to account for the observed frequency shifts and impedance spectra.^[24–29] Furthermore, the development of special oscillator circuits was necessary to cope with the high damping with liquid loads. Since the theoretical description of composite loading is common in life science problems and biosensor applications are of paramount interest, the theoretical framework developed by Bandey et al.^[25] has been chosen in this Review to explain some of the most relevant load situations in bioanalytics.

Starting with the three-port-Mason model (Figure 6 A) the piezoelectric resonator loaded on one side can be characterized by its mechanical impedance Z_s [Eq. 5] (T_{xz} = mechanical strain in the xz direction; V_x = potential in the x direction).

$$Z_s = \left. \frac{T_{xz}}{V_x} \right|_{z=0} \quad (5)$$

The electrical impedance of the system Z_m is composed of Z_m^0 [Eq. (6a)], the impedance of the unperturbed resonator, and a term Z_m^1 [Eq. (6b)] representing the load (ω = angle frequency).

$$Z_m^0 = \frac{1}{i\omega C_0} \left(\frac{kd_q/K^2}{2 \tan(kd_q/2)} - 1 \right) \approx R_q + i\omega L_q + \frac{1}{i\omega C_q} \quad (6a)$$

$$Z_m^1 = \frac{kd_q(Z_s/Z_q)}{4K^2\omega C_0} \left(1 - \frac{i(Z_s/Z_q)}{2 \tan(kd_q/2)} \right)^{-1} \approx \frac{n\pi}{4K^2\omega_s C_0 Z_q} \quad (6b)$$

K is the electroacoustic coupling factor. The approximations on the right hand side are only valid for low-load conditions of the quartz ($Z_s/Z_q < 2 \tan(kd_q/2)$). If $Z_s/Z_q \leq 0.1$ the impedance of the loaded resonator can be expressed as a lumped element equivalent circuit with Z_m^1 (Figure 10) as an additional element in series to the motional branch of the BVD-equivalent circuit (Figure 6 B). The mechanical impedance Z_s is a complex number in which the real part represents mechanical energy losses whereas the imaginary part stands for mechanical energy storage at the surface. Table 3 shows the impedance elements of Z_s for various surface coatings.

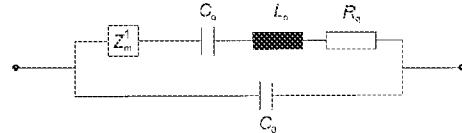


Figure 10. Modified BVD-equivalent circuit with an additional complex impedance Z_m^1 representing the load on the surface.

Table 3. Mathematical expressions of motional inductance L_L and resistance R_L for particular loads on AT-cut quartz resonators.^[25]

Load	L_L	R_L
rigid mass	$\frac{n\pi}{4K^2\omega C_0 Z_q} \rho_s$	0
Newtonian liquid	$\frac{n\pi}{4K^2\omega C_0 Z_q} \sqrt{\frac{\rho_L \eta_L}{2\omega}}$	$\frac{n\pi}{4K^2\omega C_0 Z_q} \sqrt{\frac{\omega \rho_L \eta_L}{2}}$
semi-infinite	$\frac{n\pi}{4K^2\omega C_0 Z_q} \left[\frac{\rho_v (G - G')}{2} \right]^{1/2}$	$\frac{n\pi}{4K^2\omega C_0 Z_q} \left[\frac{\rho_v (G + G')}{2} \right]^{1/2}$
viscoelastic layer		

Equations (6a) and (6b) enables one to describe typical load situations such as thin rigid films, viscous liquids, and viscoelastic polymers.^[25, 30, 31] Multilayers consisting of different kinds of surface layers can be described as a linear combination if interaction terms occurring between the different materials can be neglected. Nonlinearities will be discussed in more detail later. Three relevant cases, which are particularly interesting for applications in life science, are

thoroughly discussed in the following section: a) thin rigid films covered by a Newtonian liquid, b) semi-infinite viscoelastic solids, c) thin viscoelastic solids covered by a Newtonian liquid.

2.3.1.1.1. With a Newtonian Liquid Covering Thin Rigid Films

Covering a sufficiently thin film^[32] with a semi-infinite Newtonian liquid permits a linear combination of the mechanical impedances of the rigid mass and the liquid (Figure 11).

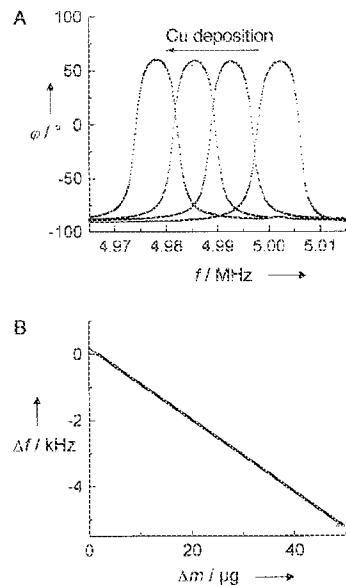


Figure 11. A) Impedance spectra of a 5 MHz AT-cut quartz before and after sequential deposition of 79, 158, and 237 μg of copper. The measurement was performed with one side of the quartz in contact with water. B) Change in the resonance frequency with increased amount of deposited copper.

The mechanical impedance of a rigid film of mass per unit area ρ_s can be expressed by $Z_s = i\omega\rho_s$. Z_s is purely imaginary, which means that the mass vibrates in phase with the resonator surface. The mechanical impedance Z_s of a liquid with viscosity η_L and density ρ_L can be determined from the velocity profile of a laminar flow parallel to the surface of the crystal [Eq. (7)], where $\delta = \sqrt{2\eta_L/(\omega\rho_L)}$ is the decay length of the damped shear wave within the fluid.

$$v_s(z,t) = v_{s,0} e^{-z/\delta} \cos(z/\delta) e^{i\omega t} \quad (7)$$

The shear wave propagates deeper into the medium with increasing kinematic viscosity (η_L/ρ_L). For instance, the decay length of a 5 MHz quartz is 250 nm in water. Assuming a mass load of a Newtonian liquid with a mass of $\Delta m = A\rho_L\delta$, Equation (3) results in the well-known expression found by Kanazawa and Gordon^[29] [Eq. (8)], who related the product of the density and viscosity of the liquid to the frequency shift of the shear wave resonator.

$$\Delta f = -f_0^{3/2} \sqrt{\frac{\eta_L \rho_L}{\pi \rho_q \bar{c}_{66}}} \quad (8)$$

Kanazawa and Gordon did not consider the damping of the resonator from viscous loading. However, the electromechanical model of Martin and co-workers^[17, 25] shows that the resistance R_L and the inductance L_L both exhibit a proportionality to the square root of the density-viscosity product of the corresponding liquid (Figure 12 A and B).

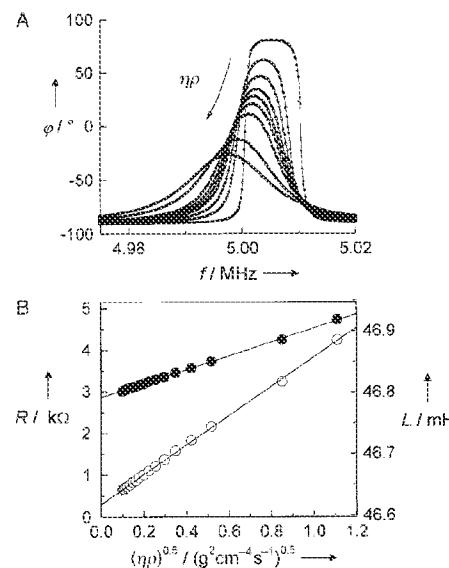


Figure 12. A) Influence of various glycerol–water mixtures on the phase shift. The solid lines are the results of fitting the parameters of the BVD-equivalent circuit. The measurements were taken in air (spectrum with the largest phase maximum), in water, as well as with glycerol–water mixtures of 40, 55, 60, 65, 70, 80, 85% glycerol (the arrow indicates an increasing amount of glycerol in solution).^[167] B) Dependence of the resistance R (○) and inductance L (●) on the square root of the density-viscosity product of various glycerol–water mixtures. The parameters were fitted using the generalized BVD-equivalent circuit composed of L , C , R , and C_0 . The capacitance C (21.655 fF) can be calculated from the intrinsic properties of the unloaded quartz (5 MHz).

A thin layer of a rigid mass covered by a semi-infinite layer of Newtonian liquid yields a linear combination, that is, addition of both impedances. The corresponding elements R_L and L_L can be obtained from a linear combination of the expressions shown in Table 3. Impedance spectra illustrating the situation of sequentially deposited copper films on a quartz crystal vibrating in aqueous solution are shown in Figure 11. Mass deposition of thin rigid films in a Newtonian liquid at constant density and viscosity can be described by Sauerbrey's equation without changes in R_L (Figure 11B).

2.3.1.1.2. Semi-Infinite Viscoelastic Solids

The semi-infinite viscoelastic solid is characterized by the absence of reflections at the air–solid interface of the shear wave traveling within the viscoelastic body. The decay length is smaller than the thickness of the solid. Therefore, wave propagation is unidirectional within the viscoelastic solid of density ρ_v , which leads to a simple expression for the mechanical impedance Z_s [Eq. (9)].^[25]

$$Z_s = \sqrt{G\rho_v} \quad (9)$$

G is the complex shear modulus, in which $\text{Re}(G)$ is the storage modulus G' and $\text{Im}(G)$ the loss modulus G'' . Both G' and G'' can be obtained from the expression of R_L and L_L . Using a specific model for viscoelastic solids, such as the Kelvin or Voight model, one can calculate viscosity and elasticity, respectively. Figure 13 shows the frequency response and the shear displacement for different viscoelastic materials.

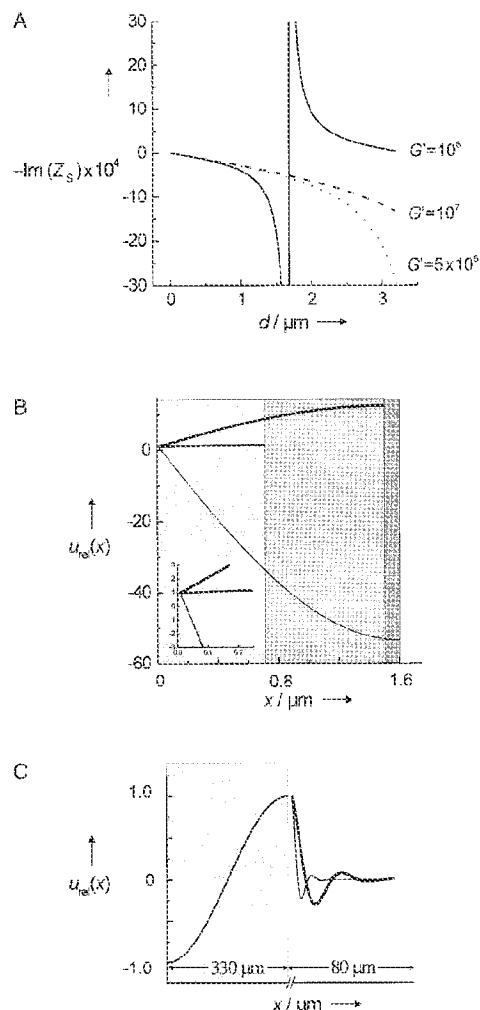


Figure 13. A) Numerical simulations of the imaginary part of the mechanical impedance (proportional to the frequency change) of a lossless viscoelastic film with different film thicknesses. An increase of film softness, namely, the shear modulus G becomes smaller, and leads to a considerable deviation from the Sauerbrey Equation even at small film thicknesses. For very soft films, even the resonance of the film is visible in the observed film thickness range, which starts at $\phi = \pi/2$ and is characterized by a sudden increase in the resonance frequency. B) Shear vibration amplitude within the film. Within the linear region of $\text{Im}(Z_s)$ versus d the polymer surface oscillates in phase with the quartz and the damping is almost zero (middle curve). With larger thicknesses an overshoot of the displacement at the film/air interface occurs; the surface of the elastic film does move synchronously with the quartz oscillation but with a higher amplitude (top curve). Immediately after resonance the oscillation of the film is 90° behind the oscillation of the vibrating quartz (bottom curve). C) Within an infinite extended viscoelastic solid the shear oscillation propagates with different wave and decay lengths. The situation depends on the complex shear modulus G and the density of the solid.

2.3.1.1.3. With a Newtonian Liquid Covering Thin Viscoelastic Solids

The combination of a sufficiently thin viscoelastic film covered by a semi-infinite layer of a Newtonian liquid cannot be treated as a simple linear combination of the individual mechanical impedances as was valid for the two previous examples. Instead, interaction terms between the two different materials have to be taken into account. Granstaff and Martin^[26] derived a general recursion formula [Eq. (10)] for a layer system with n different viscoelastic materials.

$$Z_s = Z_s^{(n)} \frac{Z_s^{(n+1)} \cosh(\gamma^{(n)} d^{(n)}) + Z_s^{(n)} \sinh(\gamma^{(n)} d^{(n)})}{Z_s^{(n)} \cosh(\gamma^{(n)} d^{(n)}) + Z_s^{(n+1)} \sinh(\gamma^{(n)} d^{(n)})} \quad (10)$$

γ denotes the complex wavenumber ik and $Z_s^{(n)}$ is the characteristic impedance of the n th viscoelastic layer. The procedure for evaluating the mechanical impedance of single layers is to start with the knowledge of the characteristic impedance of the top layer (stress free). Then, one has to work backward toward calculating the surface mechanical impedance at the resonator–film interface. This process allows the influence of many viscoelastic layers on the surface of the TSM resonator to be estimated, although extracting physical properties becomes cumbersome with increasing amount of layers. One example is a thin viscoelastic film of thickness d covered by a liquid. A description with discrete impedance elements is permitted because of the phase lag of the shear oscillation at the surface of the viscoelastic film with respect to the resonator–film interface. Resonance of the viscoelastic solid is reached at an acoustic phase shift $\phi = \omega d \text{Re} \sqrt{\rho_v/G}$ of $\pi/2$ accompanied by a sudden increase in the resonance frequency and damping of the system (Figure 13). At $\phi > \pi/2$ the surface of the film oscillates at 90° to the surface of the resonator, and exhibits higher amplitude (overshoot) than the quartz material, which is almost critically damped.

2.3.1.1.4. Conclusion

The previous examples demonstrate that the operation of TSM resonators in liquids raises theoretical and experimental problems. Besides the difficult evaluation of multilayers composed of different viscoelastic solids or fluids, contributions from the electrolyte, roughness of the surfaces, surface energy of the outermost layer, and influence of compressional waves have to be considered. Some of the most important aspects are discussed in more detail below.

2.3.1.2. Longitudinal waves

Longitudinal or compressional waves can readily be observed by filling a measuring chamber equipped with a TSM resonator at the bottom and opened to air with a volatile liquid. Periodical changes in the resonant frequency can be recorded (Figure 14) as a result of the evaporation of the liquid. Closing the chamber, however, abolishes the observed instability instantaneously.

This observation can be explained in terms of compressional wave generation. The evaporation of the liquid with time results in the height of the liquid varying, and therefore constructive and destructive interference of the compres-

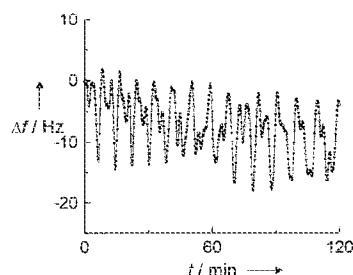


Figure 14. Periodic changes in the resonance frequency of a 5 MHz quartz immersed in ethanol. As ethanol evaporates over time, the resonance condition of the longitudinal wave, which is reflected at the ethanol–air interface, alters continuously.

sional waves reflected at the air–liquid interface occurs. This process results in a typical standing wave pattern. The origin of longitudinal waves can be reasoned by the occurrence of longitudinal or flexural modi or as shown by Martin and Hager^[23] by a nonzero velocity gradient of the shear oscillation along the x -axis (Figure 15).

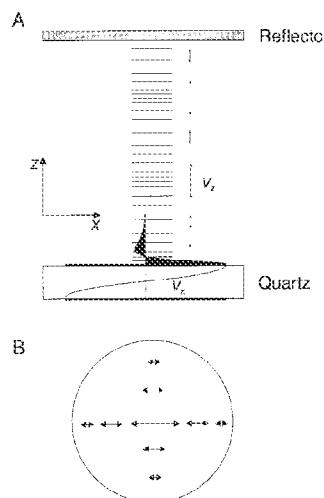


Figure 15. A) Representation of compressional waves generated in the fluid above the quartz crystal induced by a velocity gradient of the particle displacement, which are reflected at the glass surface and form a standing wave. B) Velocity profile of the particle movement on the quartz surface.^[34]

Lin and Ward^[33] as well as Schneider and Martin^[34] demonstrated by a simple experiment and theoretical treatment of the problem that indeed compressional waves are generated from the quartz surface and can be reflected at an interface. They mounted a reflector (glass plate), adjustable to the desired distance from the quartz plate (z -direction), several 100 μm away from the resonator. Both, the resonance frequency and the damping resistance R varied periodically with the distance of the reflector from the shear resonator. The periodicity was $\lambda_c/2$, in which λ_c represents the wavelength of the longitudinal wave in solution.

2.3.1.3. Conductance and Permittivity of the Solution

Other important parameters that influence the resonant frequency of the quartz crystal in solution are the ionic

strength and dielectric constant of the electrolyte. Supposing that the operator of the QCM has to change the buffer conditions for a particular experiment an undesired parasitic frequency shift may occur, which can be more or less of the same magnitude as the measuring signal itself. This effect strongly depends on the shape of the electrodes and conductance of the solution. The series resonance shows only a minor response to changes in conductance and permittivity,^[35] since all effects parallel to the motional branch add on the parallel resonant frequency of the crystal.^[36] The parallel resonant frequency decreases with increasing conductance of the solution. An expanded BVD-equivalent circuit which explained the observations was first introduced by Shana and Josse.^[37]

An additional parallel RC circuit (Figure 16B) has to be introduced to account for the influence of the conductance and dielectric constant of the medium. R_G can be neglected at high ionic strength, thus C_G and $C_{0,A}$ can be lumped into one capacitance to give the well-known BVD circuit with a modified capacitance C_0 . Rodahl et al.^[38] investigated the

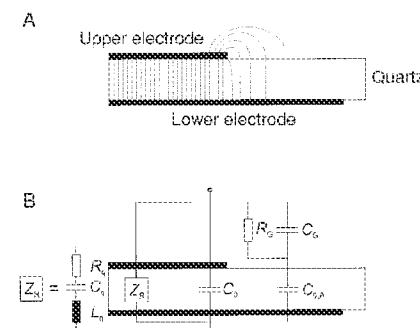


Figure 16. A) Course of the field lines at the electrode edges of a quartz oscillator. The origin of the changes in the quartz resonance frequency upon changing the ion conductance of the solution are field-fringing effects. B) These edge effects of the electric field can be represented by a RC element (R_G and C_G). As a result of the overlap of the electric field the effective area of the quartz is larger, hence the capacitance C_0 of the quartz is also increased by $C_{0,A}$.^[37, 38]

influence of the conductance on the series and parallel resonant frequency as well as the damping of the resonator using differently shaped electrodes.^[39] They found that field fringing is the predominant reason for changes in the resonant frequencies (Figure 16A). The extent of field fringing strongly depends on the shape of the electrodes. The strongest dependence of the parallel resonant frequency on the conductance of the electrolyte solution was seen for ring-shaped working electrodes in which the center of the quartz resonator is not layered, for instance, while completely covered quartz plates do not display significant frequency shifts with changing conductivity.

2.3.1.4. Surface Roughness

Interpretation of adsorption phenomena is strongly influenced by the surface roughness of quartz resonators. In particular an alteration in the hydrophilicity upon adsorption can lead to tremendous changes in the resonant frequency. Rough and hydrophilic surfaces entrap liquids in small

cavities thus contributing to the overall mass detected by the device.^[40, 41] Hydrophobic cavities, however, are often not wetted by the liquid and result in the inclusion of air or vacuum (Figure 17),^[41] thus leading to smaller energy losses on hydrophobic surfaces than hydrophilic ones. This observation implies that the resonant frequency shifts to smaller values when changing from a hydrophobic to a hydrophilic surface. Hence, smooth surfaces are required when operating in fluids so as to avoid frequency shifts arising from changes in surface energies.

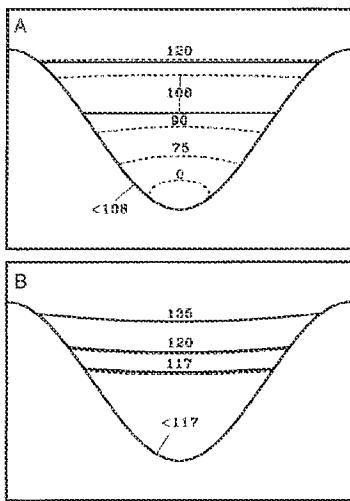


Figure 17. Cross-section of different liquid menisci formed in a sinusoidally textured surface illustrating contact angle dependent trapping (A) with trapped air and (B) without trapped air. The dashed lines indicate the initial penetration of liquid for the indicated microscopic contact angle. The solid lines indicate the equilibrium penetration, which becomes complete at $<108^\circ$ (A) and $<117^\circ$ (B).^[7]

2.3.1.5. Electrochemical Double Layer

Tsionsky et al.^[42] presented a consistent treatment of the influence of the electrochemical double layer on the resonant frequency. Considering the electrochemical double layer as a rigid mass on the basis of its extension of a few nanometers in the z -direction leads to an additional frequency change Δf_{DS} . The thickness of the double layer can be estimated to be about 1 nm for a 1:1 electrolyte of 0.1 M. By taking into account that the extension of the shear wave of a 5 MHz quartz is 250 nm in water and therefore exceeds by far the thickness of the double layer this approximation holds for most cases. The influence of a thin rigid mass oscillating in phase with the resonator surface can be expressed by Sauerbrey's Equation. A change of the electrochemical double layer as a result of additional charges adsorbing to the surface leads to the following change in resonant frequency [Eq. (11 a) and (11 b)].

$$\Delta f_{\text{DS}} = -\frac{2f_0^2}{A\sqrt{c_{66}\rho_q}}\Delta m_{\text{DS}} \quad (11\text{a})$$

$$\Delta m_{\text{DS}} = \frac{q_+}{F}(M_A - M_W n_A) - \frac{q_-}{F}(M_K - M_W n_K) \quad (11\text{b})$$

M_A and M_K are the molecular masses of the corresponding anions and cations, M_W the molecular weight of water, n_A and

n_K denote the number of substituted water molecules, and q_- and q_+ are the excess charges on the surface. Adsorption of positively charged particles on the resonator surface leads to an increase in adsorbed mass if it is assumed that sufficiently large counter ions are near the quartz. Therefore, charged particles can cause larger frequency shifts than uncharged ones. The influence of charges on the hydrodynamic properties of the adjacent water layer has not been considered yet.

2.3.1.6. Conclusion

In conclusion, interpretation of QCM data is not unambiguous and can lead to controversial results. The factors mentioned in Sections 2.3.1.2–2.3.1.5 have to be taken into account if conditions such as hydrophilicity, surface charges, meniscus, temperature, viscosity of the liquid, and buffer composition cannot be preserved. The QCM in fluids is not a simple mass-sensor but provides valuable information about interfacial reactions. Besides elasticity and viscosity, information about surface charges of biomolecules can be obtained. The following section gives an overview of other acoustic resonators.

2.3.2. Surface-Acoustic-Wave (SAW) Sensors

Lord Rayleigh was the first to discovered this acoustic mode known as surface acoustic waves (SAW). The stress-free boundary of a solid gives rise to these surface-confined waves that propagate as coupled longitudinal and transversal waves. The frequencies of SAW sensors are usually between 50 MHz and a few GHz.^[7] The displacement components decay exponentially within the solid. The surface acts as an acoustic waveguide. Surface acoustic waves can be excited and detected by patterned interdigital transducers (IDT, see Figure 3) on the surface of single piezoelectric crystals such as quartz, lithium niobate, or lithium tantalate. Each "finger" is the origin of a surface acoustic wave. The transducer works most efficiently if the periodicity matches the wavelength of the surface wave occurring at $f = v_p/d$, in which v_p denotes the propagation velocity and d the distance between the interdigital fingers, that is, the periodicity. Generally, resonators can be distinguished as two port delay lines and one-port resonators.^[43] Two port delay lines work with one IDT as a transmitter and one as a receiver. The separation between them determines the delay between the transmission and receiving of the surface wave. One-port resonators consist of one IDT structure in between two reflectors thus producing a standing wave in both directions. The resonant frequency is given by $f_n = n v_p / 2l$, in which l is the distance between the reflectors. The quality factor Q of SAW devices is between 6000 and 12000 and is considerably lower than that of TSM resonators operating in air (20 000–50 000 for a 10 MHz fundamental frequency).

2.3.2.1. Mass Loading

If the deposited mass is a thin rigid film the kinetic energy of the synchronously vibrating system is increased without any energy loss arising from viscous damping. This then leads to a

decrease in the propagation velocity. The influence on the resonant frequency is given by Equation (12).^[7]

$$\frac{\Delta f}{f} = \frac{\Delta v}{v_p} = -c_f^{\text{SAW}} f \rho_s \quad (12)$$

This equation resembles Equation (3). Again the mass sensitivity increases with the square of the fundamental frequency, which is considerably higher (> 100 MHz) than that of TSM resonators, and renders the sensitivity of a 100 MHz SAW sensor 200 times higher than the mass sensitivity of a 5 MHz quartz crystal. However, bioanalytic applications require operation in water, thus SAW sensors are less suited than TSM resonators as a result of the high energy loss occurring in an aqueous environment. Damping is a consequence of compressional waves generated by displacement components parallel to the surface normal and the viscous coupling of displacement components parallel to the surface.

2.3.3. Acoustic-Plate-Mode (APM) Sensors

APM sensors are cut from single-crystalline quartz to serve as acoustic waveguides that are particularly suited for operation in liquids. The acoustic wave is confined between the upper and lower surfaces of the plate propagating between input and output transducers, in contrast to SAW sensors in which they are restricted to one surface.^[44] Shear-horizontal (SH) APM resonators do not exhibit displacement components parallel to the surface normal but predominantly display displacement parallel to the surface and the direction of wave propagation. Therefore, loss of acoustic energy from interaction with the environment is drastically reduced. The particle displacement has only one component.^[45] SH modes can be considered as a superposition of plane waves with an in-plane (shear horizontal) displacement reflected at a particular angle between the upper and lower face of the quartz resonator. Similar to SAW sensors, acoustic waves can be excited and detected by lithographically patterned IDT metal structures (Figure 3). The input transducer with periodicity b and thickness d generates approximately the eigenfrequency calculated by Equation (13).^[45]

$$f_n = \frac{v_p}{b} \left[1 + \left(\frac{nb}{2d} \right)^2 \right]^{1/2} \quad (13)$$

2.3.3.1. Mass Loading

When the mass is strongly bound (nonslip boundary condition) to the surface the layer moves synchronously with the quartz surface. As a consequence the kinetic energy increases and the propagation velocity decreases as discussed previously for TSM and SAW resonators [Eq. (14)].^[7]

$$\frac{\Delta f}{f} = \frac{\Delta v}{v_p} = -c_f^{\text{APM}} \rho_s \quad (14)$$

c_f^{APM} denotes the mass sensitivity, and ρ_s the surface mass density (mass/area on face) of the foreign mass layer.

2.3.4. Flexural-Plate-Wave (FPW) Resonators

FPW resonators are thin, rectangular membranes made of tension-free silicon nitride embedded in a frame of silicon that are manufactured photolithographically. Oscillations of these plates, which are only a few micrometers thick, can be excited piezoelectrically by IDT, electrostatically, or by using magnetic transducers. FPW resonators are characterized by a high quality factor and low energy loss in fluids at a low resonant frequency. Although the mechanical amplitude is rather high (100 nm) energy dissipation is low since the phase velocity of the acoustic wave is lower than the velocity of sound in most liquids ($900 - 1500$ m s⁻¹).^[7] The low resonant frequencies (1 – 10 MHz) permit the use of low-cost electronics, thus providing an attractive alternative to the less sensitive TSM resonators. FPW devices can also be used as actuators for granular solids as a result of their high amplitude.

The simplest case of an oscillating isotropic plate includes an infinite set of waves known as Lamb waves.^[46, 47] Two sets of waves can be distinguished: symmetric waves (S), with particle displacements symmetric about the neutral plane, and antisymmetric waves (A), whose displacements have odd symmetry. Only two waves, A_0 and S_0 (both of lowest order), occur in sufficiently thin plates. Notably, the phase velocity of the two waves differs tremendously. Thinner membranes exhibit lower phase velocities for the A_0 mode, in which the plate undergoes flexing as the wave propagates, while the phase velocity reaches its maximum value for the S_0 mode. In the case of the A_0 mode the eigenfrequency decreases with decreasing thickness of the membrane at a given wavelength λ [Eq. (15)].^[7]

$$f = \frac{1}{\lambda} \frac{\sqrt{B}}{M} \quad (15)$$

B denotes the bending stiffness of the membrane^[48] and M the specific mass of the membrane per unit area.

2.3.4.1. Mass Loading

Increasing the mass of the isotropic plate by a thin rigid layer of foreign mass results in a decreased phase velocity of the A_0 Lamb wave [Eq. (16)].^[7]

$$\frac{\Delta f}{f} = \frac{\Delta v}{v_p} = -\frac{\rho_s}{2M} \quad (16)$$

The integral mass sensitivity $-1/2M$ can be increased by using thinner plates, thus reducing the phase velocity and hence the resonant frequency. A comparison of the different acoustic resonators is given in Table 4.

3. Adsorption of Biomolecules and Cells

3.1. Quartz-Crystal Microbalances

The core component of a quartz-crystal microbalance is the AT-cut quartz plate with fundamental resonance frequencies predominately in the range of 5 – 30 MHz. Since quartz plates with high fundamental frequencies are very thin and therefore difficult to handle, most quartz plates in use have fundamental

Table 4. Comparison of the different acoustic resonators. d = plate thickness; λ is the wavelength of the acoustic wave.

Resonator	d	Medium	f [Hz]	Example	Temperature stability	Mass sensitivity S_m ^[a] [(Hz/MHz)(ng/cm ²) ⁻¹]
TSM	$\lambda/2$	g, fl	4–30	quartz 6 MHz, $d = 277 \mu\text{m}$ $v_p = 3330 \text{ m s}^{-1}$, $\lambda = 554 \mu\text{m}$	high	0.014 (6 MHz)
SAW	$\gg \lambda$	g	30–500	quartz 158 MHz, $d = 760 \mu\text{m}$ $v_p = 3160 \text{ m s}^{-1}$, $\lambda = 20 \mu\text{m}$	high/medium	0.20 (158 MHz)
SH/APM	$3–10\lambda$	g, fl	25–200	quartz 101 MHz, $d = 203 \mu\text{m}$ $v_p = 5060 \text{ m s}^{-1}$, $\lambda = 50 \mu\text{m}$	high	0.019 (101 MHz)
FPW	$\ll \lambda/2$	g, fl	2–7	ZnO 5.5 MHz, $d = 3.5 \mu\text{m}$ $v_p = 550 \text{ m s}^{-1}$, $\lambda = 100 \mu\text{m}$	medium	0.38 (5.5 MHz)

[a] $S_m = \frac{\Delta m \rightarrow 0}{(\Delta f/f)1/\rho_s}$.

resonance frequencies in the range of 4–10 MHz. Overtones are excited in order to obtain resonance frequencies larger than 15 MHz. Despite mechanical problems, very sensitive quartz crystals with fundamental resonance frequencies of about 30 MHz are also used.^[49]

For the analysis of biomolecules and cells, measurements in fluids are required—particularly in aqueous solutions. By developing appropriate oscillator circuits capable of exciting AT-cut quartz crystals to their resonance frequencies under liquid load,^[50, 51] the quartz crystal microbalance could be introduced as a powerful tool in life science. Up to now several quartz crystal microbalance setups have been realized:

- A flow system comprising a quartz plate that is usually clamped between two O-rings. Since the radial mass sensitivity, described by a Gaussian function, decays towards the edges of the quartz plate it is advisable to minimize the contact area of the O-ring with the resonator and to place it as far outside the quartz as possible, so that damping is minimized and thus the quality factor is at a maximum. The measurement chamber has to be sealed to air to avoid air bubbles and alteration of the liquid meniscus in order to avoid reflection of induced longitudinal waves at the air–liquid interface which influence the resonance frequency of the quartz. An outlet and inlet of the quartz holder allows the addition of analytes at any given time while monitoring the resonance frequency. Apart from thermodynamic equilibrium values, kinetic data can also be obtained with this setup. The time resolution of the quartz crystal is limited by its quality factor and is in the range of milliseconds. Using a flow system ensures a proper mixing of the solution, so that rate-controlled kinetics might be assumed. Moreover, most setups can be fully automated for sensor applications.
- A different approach to excite the quartz which has only one side in contact with liquids is to seal one side of the quartz with a rubber casing. Only one side is exposed to the fluid when the quartz is completely immersed into solution, with the other one kept in air. Addition of the analyte can be performed using a syringe while stirring the solution continuously.
- Further techniques to immerse quartz crystals with only one side in aqueous solution are based on moving a quartz plate horizontally at the air–water interface; beforehand,

a film is spread at the air–water interface and compressed to the desired film pressure. In this way, the quartz plate is in close contact with the monolayer. Upon addition of the analyte into the subphase, the interaction can be monitored.

- The simplest approach is to monitor frequency shifts in air after adsorption of the biomolecules in fluids and drying of the surface. This technique allows only the determination of final frequency values with large errors arising from the continuous change between air and liquid. The resonance frequencies obtained vary considerably compared to those gathered from measurements in liquids, since the water content of the biomolecules influences the response of the quartz significantly. The only advantage of this technique is given that the Sauerbrey Equation might be valid under these conditions.
- Inspired by the finding that the vibration of the quartz is considerably influenced by a liquid, the so-called reference crystal method has been developed. With this setup, two quartz crystals are excited in parallel. One of the surfaces of the two quartz crystals is functionalized while both of them are immersed in the same medium. This results in a net detection of the biomolecules. However, this method is not frequently used since other disadvantages occur from the different surfaces of the two crystals which lead to temperature and resonance frequency instabilities.

There are basically two different modes of operation for TSM resonators. One is based on the quartz crystal being the frequency-determining element of the oscillator circuit. The surface coverage of the quartz can be obtained by monitoring the resonance frequency. The read out of the resonance frequency is routinely performed using an oscillator circuit connected to a frequency counter. This mode of operation is termed active, since the crystal is excited to its resonance frequency and the oscillator circuit compensates for the energy loss. One should keep in mind that the exact resonance frequency that is excited by the oscillator circuit is not necessarily known so that experimental results might not be comparable using different oscillator circuits. Especially, if energy dissipation as a result of viscoelastic load occurs then frequency responses can be quite different. The equations depicted in Table 2 outline the various resonance frequencies of a moderately damped system to demonstrate this problem.

Therefore, it is important to know whether the serial or parallel resonance is supported by the oscillator circuit. However, the feedback of the oscillator circuit often introduces inevitable phase shifts, which result in considerable deviations from the serial or parallel resonance so that an accurate determination of the excited resonance frequency remains difficult.

The other mode of operation, the so termed passive method, uses a network analyzer/frequency generator to excite the crystal to a constraint vibration near resonance while monitoring the complex electrical impedance or admittance dependent on the applied frequency. By fitting the parameters of the BVD-equivalent circuit to the spectra (Figure 6B) both the mass load and energy dissipation can be determined separately.

A connecting link between simple frequency determination and complex network analysis is the so-called QCM-D technique developed and commercialized by Kasemo and co-workers,^[52] which allows the resonance frequency and dissipation factor to be monitored simultaneously. In this technique a quartz plate is excited with a frequency generator followed by switching off the source and recording the free decay of the quartz oscillation. This procedure is repeated each second. The dissipation factor together with the resonance frequency is obtained by a curve fit. Damping can also be recorded using an amplitude-controlled oscillator circuit that monitors the amplitude separately from the resonance frequency.^[53]

3.2. In Situ Hybridization of DNA/RNA on Quartz Surfaces

The analysis of genetic material, such as that essential for the diagnosis of hereditary and infectious diseases, for the classification of an organism, and in the field of forensic chemistry, has attained enormous importance and has led to numerous techniques to quantify nucleic acids in a sensitive, selective, and fast manner, that is, high-throughput screening. In the course of these developments mass-sensing devices were included in the repertoire of signal transducers that are capable of detecting oligonucleotides label-free and online. The first evidence of a direct measure of nucleic acids using an acoustic resonator was given by Fawcett et al.,^[54] however, the experiments were performed in air. The first *in situ* experiment was accomplished in fluids using an acoustic-plate-mode resonator^[44] and the second one with a thickness-shear-mode resonator, which was functionalized with a single-stranded DNA coupled through a self organized 11-sulfanylundecanol monolayer chemisorbed on gold.^[55]

The crucial step in developing a mass-sensitive nucleic acid detecting device is the immobilization of a single-stranded oligonucleotide on the resonator surface which hybridizes selectively with the complementary strand from solution. Well-known procedures are the modification of 5'-phosphate residues by thiol groups.^[56–60] In this way, DNA as well as RNA can be immobilized, and also peptide nucleic acids carrying a polyethylene-modified terminal cysteine.^[61] Suitable procedures solely for DNA and RNA are based on

electrostatic interactions of the negatively charged backbone and positively charged amine monolayers.^[62, 63] Frequently used methods, though quite complicated, are based on the binding of biotinylated oligonucleotides to surface-confined avidin or streptavidin. The coupling of proteins is accomplished by simple physisorption,^[64] by electrostatic interaction,^[65] or covalently linked through an amide bond between chemisorbed 3,3'-bispropionic acid and the amine residue of the protein.^[56, 65, 66] Unfortunately, the oligonucleotide coverage is not well determined in most cases; surface coverages are mostly calculated after adsorption of the nucleic acid in air or liquid using the proportional constant of the quartz as determined before. The average values obtained are well below 100%, with typical coverages between 10 and 30%. A correct correlation between the actual concentration of the nucleic acid at the surface and the frequency decrease, as experimentally determined by Su et al.,^[67] demonstrated that the surface coverage obtained from the frequency shift is 3 to 12 times larger than that determined by radioisotope labeling. The question is whether the common oligonucleotide immobilization techniques provide satisfactory surface coverages. The quality of the immobilization method is rather important for dealing with aspects such as sensitivity and specificity since the surface coverage reflects the sensitivity of the mass-sensing device. Moreover, nonspecific adsorption can be minimized by complete surface coverage. A desirable, user-demanded detection limit is approximately 10^{-18} M, but depends on the number of base pairs.^[68] In order to minimize the detection limit of a functionalized resonator surface quartz crystals with higher resonance frequencies were used, multilayers composed of nucleic acids and polymers/proteins were developed.^[63] DNA dendrimers were synthesized,^[69] and nucleic acids were amplified on the surface by using the polymer chain reaction (PCR).^[70] In a recent study Bardea et al.^[71] used anti-double-stranded DNA and anti-mouse F_c antibodies as a second antibody to amplify the signal. Despite these efforts, the detection limit is currently in the range of 10^{-8} – 10^{-7} M.^[56, 60, 69]

Besides difficulties of nucleic acid immobilization and sometimes unknown surface coverage the reason for a low detection limit can also be sought in the low hybridization yields on the surface, which do not exceed 10%.^[57, 59, 67] Steric hindrance of the hybridization reaction explains this low value; however, the equivalence of mass change and frequency upon the detection of nucleic acids in solution is questionable. Although the translation of the frequency shift into mass using the Sauerbrey Equation is pursued in many publications, one should refer to the articles of Thompson and co-workers^[67, 72–74] and Fawcett et al.,^[68] who showed that oligonucleotides immobilized on the resonator surface in fluids do not behave like an ideal rigid mass. Thompson and co-workers revealed, for example, that the serial resonance f_s upon binding of DNA exhibits a frequency change which is 18 times larger than predicted by the Sauerbrey Equation. They attributed this discrepancy to an altered viscosity on the surface that was generated by hybridization. Network analysis revealed that the motional resistance R , which is indicative of energy loss, changes in the same fashion as the serial resonance frequency. For immobilized DNA, R is not only

dependent on the liquid's viscosity but is also influenced by the electrolyte effecting the electroacoustic coupling of the oscillation. Thus, R is additionally determined by the solution's conductivity and the electrochemical double layer, which makes a particularly large contribution in the case of highly charged DNA.^[67] These altered viscoelastic effects can be used to obtain kinetic data of the DNA hybridization on the surface.^[72] However, a systematic study remains to be performed.

Besides sensitivity, the specificity of oligonucleotide biosensors is also of paramount importance. Bardea et al.^[60, 71] showed, that a seven base pair mutation within a 31-mer that occurs in the genome of the Tay–Sachs disease is sufficient to inhibit hybridization of the mutated DNA with surface-immobilized complementary strands. In contrast, 10-mers containing two terminal mutations hybridize almost completely (92%), while DNA with one mutation positioned in the center of the strand hybridizes with a yield of only 30%. However, nonspecific adsorption of oligonucleotides could not be excluded.^[57] An increased sensitivity was gained by using peptide nucleic acids instead of DNA on the surface. Functionalization of gold surfaces with peptide nucleic acids has the effect that a single point mutation of the complementary DNA strand is sufficient to inhibit DNA binding to the surface (Figure 18).^[61]

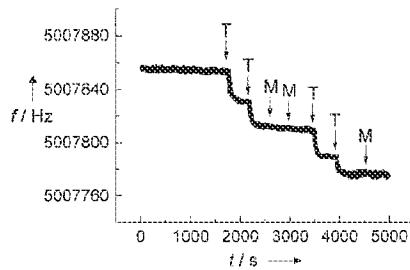


Figure 18. Frequency-time response of a QCM biosensor functionalized with a peptide nucleic acid of the sequence Cys-egl-GGC-AGT-GCC-TCA-CAA for multiple additions of $10 \mu\text{g mL}^{-1}$ of the 15-mer 5'-TTG-TGA-GGC-**A**CT-GCC-3' (T) and of $50 \mu\text{g mL}^{-1}$ of 5'-TTG-TGA-GAC-**T**CT-GCC-3' (M). The mismatch base is indicated in bold.^[61]

A recent study of Wang et al.^[75] illustrates the use of the quartz-crystal microbalance to monitor real-time enzymatic activity of RNases and DNases using surface-confined poly(U) single strands and $(\text{dA})_{20}-(\text{dT})_{20}$ double strands. The specificity of both enzymes was preserved on the surface.

3.3. Adsorption of Proteins at Functionalized Surfaces

The primary area of application of the quartz-crystal microbalance today is the investigation of protein adsorption at functionalized surfaces. For instance, the basic principle of piezoelectric sensors is the detection of the binding of antibodies to surface-confined antigens. For a long time it was postulated that a direct quantification of the adsorbed amount of protein would be feasible by using the Sauerbrey Equation. However, a number of publications established that protein adsorption performed in liquid leads to larger

frequency shifts than in air. In order that the quartz-crystal microbalance can be used as a universal tool it is desirable to find reasons for the different frequency changes. A study from 1993 found for the first time a direct correlation between mass load and frequency shift by adsorbing human serum albumin (HSA) on the resonator.^[76] Muratsugu et al. quantified the mass load by using radioisotope-labeled HSA in combination with the determination of the frequency shift by using the quartz-crystal microbalance. This result is a hallmark with respect to the sensitivity of shear-wave resonators. While $\Delta f / \Delta m$ is supposed to be $0.183 \text{ Hz cm}^2 \text{ ng}^{-1}$ for a 9 MHz quartz as predicted by Sauerbrey, the results revealed values of $(0.375 \pm 0.012) \text{ Hz cm}^2 \text{ ng}^{-1}$ for HSA and a value of $(0.716 \pm 0.066) \text{ Hz cm}^2 \text{ ng}^{-1}$ for anti-HSA. On the one hand, these numbers are considerably larger than that expected from the Sauerbrey Equation, and on the other hand, they depend on the investigated protein. In order to clarify this discrepancy, Kasemo and co-workers developed a measuring device that allowed frequency shifts to be monitored simultaneously with energy loss, which was represented as the dissipation factor with a time resolution of 1 s.^[52] Energy loss can occur within the adsorbed film, where included water also has to be taken into account, or at the interfaces as a result of friction. Their experimental results in aqueous solution confirmed that energy loss, that is, an increase in the dissipation factor, can be detected.^[77–79] Thus, binding of methemoglobin (met-Hb) and hemoglobin-CO (HbCO) on a hydrophobic methyl-terminated monolayer exhibits the expected frequency decrease as well as an alteration of the dissipation factor D (Figure 19 A, B). Plotting the frequency shift versus change in D gives rise to two distinct slopes, which indicates that a two step adsorption process occurs (Figure 19 C).

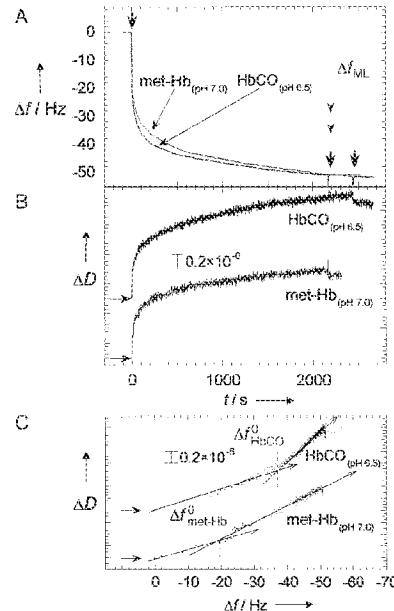


Figure 19. A) Changes in frequency and B) dissipation as a function of time during the adsorption of met-Hb and HbCO at pH 7.0 and pH 6.5, respectively, onto a methyl-terminated thiol monolayer (ML). C) Plot of the change in the dissipation factor versus the change in frequency. Two different slopes, which indicate a two-step adsorption process, are discernable for the adsorption of both met-Hb and HbCO.^[78]

These results have led to the conclusion that energy loss does not only occur at the interfaces but also in the protein layer itself; dissipation might be attributed to conformational changes within the adsorbed protein layer and tightly bound water; they are induced by periodic shear movement. The hypothesis that enclosed water contributes considerably to the frequency shift was confirmed by Rickert et al.^[80] Upon deposition of multilayers composed of alternating layers of biotinylated BSA and streptavidin they obtained a sensitivity that was four times larger than the integral mass sensitivity as predicted from the Sauerbrey Equation. By determining the thickness of the layer they could demonstrate that approximately 75 % of the overall mass is water. They excluded viscoelastic effects since a nonchanging frequency shift per protein layer up to 20 monolayers was observed. A decrease in the frequency response by a factor of approximately 0.7 would be expected according to Rickert et al. if the protein layer showed viscoelasticity.^[80]

Although the frequency shift cannot be translated into mass load, it is nevertheless conceivable that the quartz-crystal microbalance could be used for *in situ* measurements of binding events. Concentration-dependent measurements of the frequency shift together with the assumption of a linear relation between frequency shift and mass load allow the binding and rate constants of the protein and peptide adsorption to be determined.^[66, 81–85] The technique is well suited for the quality control of multilayers prepared by the Langmuir–Blodgett technique^[86] or self-organization processes.^[80, 87–94]

3.4. Lipid–Protein Interactions at Solid-Supported Lipid Membranes

Protein–receptor interactions at lipid membranes, for example ganglioside–toxin interactions play an essential role in biological processes. The first contact of a protein, virus, or bacterium with its receptor at a biological membrane initiates a variety of reactions at the cell membrane. Artificial membrane systems which are variable in their lipid composition are necessary for investigating these kind of interactions at a lipid membrane.^[95, 96] Nowadays, common model membrane systems are vesicles and black lipid membranes. Lipid membranes immobilized in a highly ordered fashion on solid supports are of great interest for the quartz-crystal microbalance. Different preparation techniques are available to prepare so-called solid-supported membranes. The Langmuir–Blodgett and Langmuir–Schäfer techniques, for example, allow the transfer of highly ordered lipid monolayers from the air–water interface onto a pretreated quartz surface. Instead of using these equipment-intensive methods, techniques based on the chemisorption of thiol or disulfide components are well suited for immobilizing lipid bilayers on gold-covered quartz plates. In a first step gold surfaces are functionalized using sulfur-containing components. These self-organized monolayers serve as starting points for the preparation of lipid bilayers. Common methods for the preparation of lipid bilayers are the vesicle-fusion technique,

detergent dilution method, or painted lipid membrane procedure.^[96]

Okahata and co-workers^[97–100] immobilized lipid monolayers at the air–water interface of a quartz surface by dipping it horizontally from the air-side at the interface. The hydrophilic head groups are oriented towards the water subphase so that proteins and peptides dissolved in the subphase may interact with them. These functionalized quartz plates allowed the binding and dissociation constants of the interaction of melittin and β -globulin with dipalmitoylphosphatidylethanolamine membranes to be determined; moreover the binding of the lectine concanavalin A to glycolipid monolayers was quantified.^[101] It is very easy to incorporate various numbers of receptor molecules in those lipid monolayers at the air–water interface. With the same preparation technique it was feasible to investigate the binding behavior of the influenza A virus and wheat germ agglutinin with G_{M3} -doped monolayers composed of sphingomyeline and/or glucosylceramide. Variation of the lipid matrix and the dopant concentration revealed that only these parameters are pivotal for virus binding and that the binding rate is influenced considerably. The system allows the inhibition of the virus binding by adding sialylactose (Neu5Ac- α -2–3Gal- β -1–4Glc).^[97, 99, 100, 102]

The above-mentioned methods based on the self-organization of lipids on surfaces make it possible to readily prepare lipid membranes that are composed of two single leaflets. A lipid bilayer composed of a first chemisorbed alkanethiol monolayer and a second lipid monolayer can be obtained by fusing unilamellar vesicles on the hydrophobic monolayer. These vesicles fuse on the surface so that their composition also determines that at the surface. Impedance analysis of the mono- and bilayers allows an exact quality control of each layer, thereby ensuring highly reproducible membrane preparations (Figure 20).

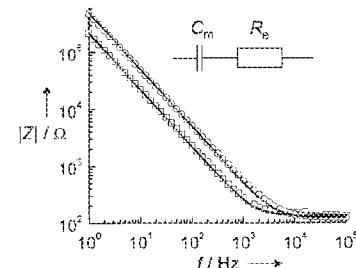


Figure 20. Impedance spectra of an octanethiol monolayer (□) and a lipid bilayer composed of octanethiol and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC; ○). The solid lines show the results of fitting the parameters of the simple equivalent circuit depicted.^[103]

A comparison of two different QCM investigations demonstrates that the choice of the artificial membrane system significantly influences the obtained results. Both studies dealt with the adsorption of melittin to phospholipid membranes. Whereas Okahata and co-workers used lipid monolayers at the air–water interface and obtained an ideal Langmuir adsorption isotherm for melittin concentrations of up to 100 μ M,^[100] Steinem et al.^[103] used lipid bilayers and

obtained adsorption isotherms which indicated multilayer adsorption started at a concentration of 8 μM . The immobilized membrane was solubilized by melittin at concentrations larger than 20 μM , these effects were not observed at the air–water interface.

In principle, the quartz-crystal microbalance in combination with lipid membranes composed of an alkanethiol monolayer and a second lipid monolayer obtained by vesicle fusion allows an easy determination of thermodynamic and kinetic parameters of protein–ligand couples without the use of labels. The example of the interaction of peanut agglutinin (PNA) with gangliosides shows the suitability of this approach. A lipid membrane is doped with different concentrations of the receptor lipid $\text{G}_{\text{M}1}$ and the frequency shift is monitored upon addition of PNA. Figure 21 shows that a dopant concentration of 1.3 mol % of the receptor lipid $\text{G}_{\text{M}1}$ is sufficient to achieve maximum coverage of the protein

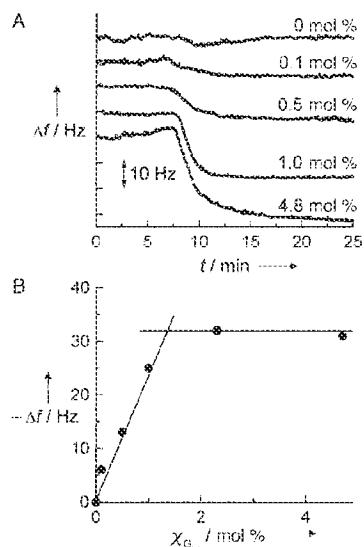


Figure 21. A) Frequency shifts of a 5 MHz quartz functionalized with solid-supported octanethiol/POPC lipid bilayers containing different amounts of $\text{G}_{\text{M}1}$ upon addition of 2 μM of PNA. B) Dependence of the equilibrium value of the resonance frequency Δf on the mole fraction $\chi_{\text{G}_{\text{M}1}}$ within the phospholipid monolayer. The solid lines intersect at $\chi_{\text{G}_{\text{M}1}} = 1.3$ mol %.^[107]

surface. A calculation of the theoretical value of the minimum number of necessary $\text{G}_{\text{M}1}$ molecules within the lipid matrix, assuming a homogenous distribution of the receptor lipids and correct values for the geometry of the protein, leads to a value of 1.5 mol %. A comparison of the theoretical value with the one obtained experimentally implies that the monomeric protein coverage on the surface has to be close to one. Similar maximum protein coverage using a lipid matrix doped with 2 mol % of the receptor lipid was corroborated by Ebato et al.^[104] who investigated the streptavidin–biotin couple with the quartz-crystal microbalance. The frequency shift Δf has to be monitored at various concentrations of the protein c_0 in solution to determine the binding constant of a protein–receptor couple (Figure 22). By assuming that the binding sites on the surface are energetically equivalent and that there is a

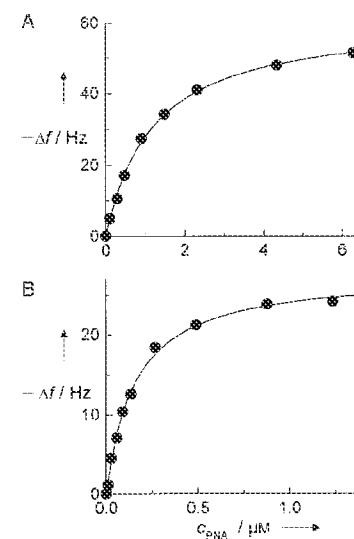


Figure 22. Isotherm of the binding of PNA to a $\text{G}_{\text{M}1}$ -doped lipid bilayer (A) and to an *asialo*- $\text{G}_{\text{M}1}$ -doped octanethiol/POPC bilayer (B) immobilized on a gold-covered AT-cut quartz. The dependence of the equilibrium resonance frequency shift Δf on the PNA concentration in solution is shown. The POPC monolayer was doped with 4.8 mol % of the receptor lipid. The solid line is the result of fitting the parameters of a Langmuir isotherm according to Equation (17) with $K_a = (0.8 \pm 0.1) \times 10^6$ and $(6.5 \pm 0.3) \times 10^6 \text{ M}^{-1}$, respectively, and $\Delta f_{\text{max}} = -(61.0 \pm 0.5)$ and (28.0 ± 0.5) Hz, respectively.^[109]

homogeneous distribution of the receptor lipids,^[105] the binding constant K_a can be obtained by fitting the parameters of a Langmuir adsorption isotherm [Eq. (17)] to the data.

$$\Delta f = \Delta f_{\text{max}} \frac{K_a c_0}{1 + K_a c_0} \quad (17)$$

The established binding constants present information about the chemical structure of the receptor essential for an appropriate binding, as demonstrated by the adsorption of PNA to $\text{G}_{\text{M}1}$ and *asialo*- $\text{G}_{\text{M}1}$. While the binding constant of PNA to $\text{G}_{\text{M}1}$ is $K_a = (0.83 \pm 0.04) \times 10^6 \text{ M}^{-1}$, it is determined to be almost a factor of 10 larger at $K_a = (6.5 \pm 0.3) \times 10^6 \text{ M}^{-1}$ for *asialo*- $\text{G}_{\text{M}1}$.^[105] This difference is attributed to the fact that *N*-acetylneuramic acid of $\text{G}_{\text{M}1}$ is not necessary for or even disrupts the binding of PNA. However, it has been demonstrated that the affinity of PNA to the trisaccharide β -Galp-(1,3)-GalNac-(1,4)- β -Galp is larger than to the disaccharide β -Galp-(1,3)-GalNac.^[106]

This is an example how the molecular structure of a receptor molecule can be illuminated by varying the receptor molecules embedded in the lipid membrane using the quartz-crystal microbalance. Besides quantifying the inhibition of binding in solution this method is capable of clarifying carbohydrate structures that play a pivotal role in receptor function. Monitoring the frequency shift upon binding of PNA to $\text{G}_{\text{M}1}$ in the absence of an inhibitor (Figure 23) allows the binding constant K_I of the inhibitor in solution to be determined.^[107] A prerequisite for the determination of K_I is an appropriate ratio between K_I and K_a . If the binding constants have similar orders of magnitude an exact determination of the binding constant K_I is practicable since the

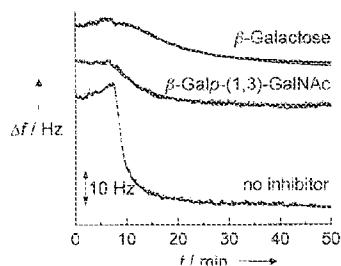


Figure 23. Time course of the resonance frequency shift upon addition of a 2 μ M solution of PNA to a lipid bilayer composed of octanethiol and POPC doped with 4.8 mol % of G_{MI} without inhibitor, with 0.27 mM β -Galp-(1,3)-GalNAc, and 26.5 mM β -galactose.^[107]

frequency changes continuously with the inhibitor concentration in solution. If there are several orders of magnitudes between K_I and K_a , the protein binds either almost unaffectedly on the surface or not at all.

The PNA–ganglioside system displays the potential of the quartz-crystal microbalance combined with solid-supported membranes in regard to studying ligand–receptor couples and the parameters which can be calculated from the obtained data. In summary, it can be concluded that the QCM technique enables one to quantify binding constants and kinetics in a relatively simple fashion so that information can be gathered about the structure of natural receptor molecules. This feature was particularly demonstrated in a study dealing with the adsorption of bacterial toxins—cholera, tetanus, and pertussis toxin—on various gangliosides.^[104, 107–109]

Besides basic knowledge that can be garnered from lipid membranes immobilized on quartz crystals, this system might also be useful for biosensor applications. Solid-supported lipid membranes on gold surfaces are not only well-suited because they can be prepared reproducibly with an exactly adjustable composition, but also because they are of particular interest, since nonspecific protein adsorption arising from the lipid matrix is strongly suppressed. First experiments pointing in the direction of regenerating the sensor surfaces after binding of a protein were demonstrated by Janshoff et al.^[107] Upon addition of protease, the adsorbed protein can again be released from the surface (Figure 24).

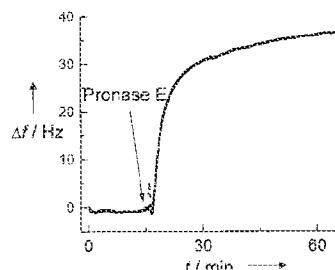


Figure 24. Time course of the resonance frequency shift upon addition of a pronase solution to a PNA-covered lipid bilayer composed of octanethiol and POPC doped with 4.8 mol % of G_{MI} . The PNA concentration was 2 μ M, the concomitant frequency shift –36 Hz. The concentration of pronase E was 0.2 % (w/w). The frequency increase arising from the action of pronase was 35 Hz after incubation of the protein monolayer in solution for approximately 1 h.^[107]

3.5. Piezooimmunosensors

The high specificity of antigen–antibody reactions and the ability to generate antibodies against a variety of biological and nonbiological substances opened up a way to develop immunosensors to address questions in areas ranging from clinical diagnosis, food control, to environmental analysis. During the last three decades radioisotope-labeled antibodies were mostly used for immunoassays.^[110] Disadvantages that emerge from work with radioactive components has led to the development of new marker systems, that is, fluorescence-labeled antibodies. Nowadays the enzyme-linked immunosorbant assay (ELISA) is the most widespread analysis tool to detect antibody–antigen reactions. The peculiarity of this detection method is the amplification of the antigen–antibody reaction using an enzyme that is bound to the antibody and catalyzes a detectable reaction, namely, the formation of a UV/Vis-active or fluorescence active dye.^[111] Despite this established technique, interest in discovering label-free and less time consuming online detection methods is still undiminished. Numerous articles were published dealing with the immobilization of antibodies or antigens on transducer surfaces whose signal is affected upon binding of the complement. Such immunosensors are valued with respect to their handling, their overall costs, and how the sensor surface can be recycled without loss of sensitivity.

Piezoelectric immunosensors, such as the quartz-crystal microbalance and SAW sensors, are suitable transducer surfaces which fulfill the above demands appropriately. Piezoelectric immunosensors in the gas phase have been investigated for quite some time^[112–116] whereas the online detection of antibody–antigen reactions in aqueous solutions originally started with the work of Roederer and Bastiaans on SAW sensors^[117] and Thompson et al.^[118] on AT-cut quartz plates. Contradictory to the classical understanding of piezoelectric sensors Thompson and co-workers found a frequency increase upon binding of immunoglobin G (IgG) to the immobilized antigen in their first study. They concluded from this result that an alteration of the microviscosity at the interface was induced upon binding of the antibody.

Similar publications from this time also dealing with the piezoelectric detection of IgG established, however, that a frequency decrease was observed upon the binding of an antibody; hence the previously published frequency increase remains questionable.^[119, 120] All further studies supported the decrease in frequency upon the binding of an analyte to a surface.

Nowadays the applicability of piezooimmunosensors to various fields has been proved. The spectrum ranges from medical applications for the detection of bacterial toxins^[121–123] and viruses^[114, 116, 124–128] through the determination of bacteria^[55, 115, 129–132] in the food industry, to environmental analysis for the detection of organic compounds by using an antigen–antibody reaction.^[122, 133–137]

For the development of a functional bioimmunosensor based on piezoelectric transducers, problems characteristic to all biosensors need to be solved: 1) functionalization of the electrode-covered surface, 2) sensitive, specific, and reproduc-

cible detection of the analyte in solution, and 3) regeneration of the sensor surface.

The immobilization technique of antigens or antibodies regularly leads not only to a functionalized sensor surface but also determines substantially the sensitivity and reproducibility of the sensor. A frequently used method is based on the simple physisorption of the desired compound on clean gold surfaces^[120, 124, 126–128, 131, 138, 139] and polymers.^[88, 115, 140] The stability of those immobilized molecules does not differ significantly from covalently coupled ones.^[129, 132, 138, 141, 142] However, problems arising from these techniques are the nondirectional orientation of the molecules on the surface, namely, that part of the antigens/antibodies is not accessible. An oriented immobilization of antibodies can be achieved by nonoriented physisorption of antibodies or covalent coupling of protein A followed by an oriented adsorption of the antibody through the F_{ab} domain. Thus, the F_{ab} domain points preferentially to the external medium.^[114, 119, 120] Another possibility is to utilize the linkage of the antibody to an individual thiol group.^[143] In this way Göpel and co-workers succeeded in the arranged linkage of an antigen—a synthetic peptide of the mouth and claw epidemic virus—on a ω -hydroxyundecanethiol monolayer.^[87, 144, 145] Another technique for the oriented immobilization of antibodies using Langmuir–Blodgett films with F_{ab} fragments bound to linker phospholipids was successfully demonstrated by Vikholm et al.^[143, 146]

However, a major problem remains: the nonspecific adsorption at the transducer surface that can only be minimized by improved immobilization techniques and cannot be influenced by the transducer itself. The functionalized surface is often blocked with a protein such as BSA or casein before the binding of the analyte so as to minimize nonspecific adsorption. Additionally, nonspecific adsorption, which is predominantly caused by hydrophobic interactions, can be reduced by the addition of detergents.^[124, 126, 128]

Apart from nonspecific interactions, which generate undesired signals independent of the choice of the transducer, viscous coupling of the liquid can cause severe problems if the solution's composition is varied during the experiment. The shear-wave resonator responds sensitively to variations in the solution's properties, namely, an altered viscosity. There are several possible ways of minimizing this bulk effect. If the viscosity of the solution under investigation is known, the viscosity of the medium can be adjusted by adding an appropriate amount of glycerol.^[128] Aberl and co-workers used diluted humane serum albumin as a medium to minimize nonspecific adsorption.^[124, 126] More general procedures to prevent this problem, which do not require the knowledge of the solution's composition, are the reference crystal method^[147] and the same-condition method.^[148] A new procedure was introduced by Zhang et al.,^[149] after they were able to distinguish the mass load of the quartz from viscoelastic effects by measuring the resonance frequency and the amplitude of the applied voltage simultaneously. The obtained frequency shift could be corrected by viscoelastic effects with this setup.

The minimization of nonspecific adsorption enhances the sensitivity of the piezoelectric immunosensor. Furthermore, the minimum concentration which can still be detected with

this method is determined by the binding constant of a given ligand–receptor couple. Ebato et al.^[135] showed that the immobilization of an antigen (fluoresceine-labeled lipid) decreases the binding constant on the surface by a factor of 300 relative to the reaction in solution.

Eventually, the question remains as to whether the surface can be regenerated after the binding of the analyte and allow further binding studies. Willner and co-workers used a reversible cleavage of antibodies which were bound to an antigen-covered gold surface of a shear-wave resonator. They functionalized gold surfaces of 9 MHz quartz plates with the photoisomerizable substrate *N*-methyldinitrospiropyran, which exhibits an affinity to the antibody antidinitrophenyl-Ab. Light with a wavelength of $360 \text{ nm} < \lambda < 380 \text{ nm}$ induced an isomerization of *N*-methyldinitrospiropyran to *N*-methyldinitromerocyanin, which does not exhibit an affinity to the antibody. The resiomerization occurs at $\lambda > 495 \text{ nm}$. The reversible binding of the antidinitrophenyl-antibody was impressive as demonstrated by OCM measurements.^[150–152] Another technique to achieve reversible antibody–antigen interactions at surfaces was presented by Sargent and Sadik.^[140] They immobilized anti-HSA antibodies on a conductive polypyrrole surface on a quartz plate and were able to induce a reversible binding by applying voltage steps.

In summary, piezoelectric immunosensors are an alternative to established ELISA methods. In contrast to ELISA techniques, which need a marker molecule and take approximately two hours, this method can be fully automated. Nowadays, the quartz-crystal microbalance provides a technique for analyzing phage libraries within a short time by using a flow injection system.^[153] The detection of the analyte can occur online and is label-free within 10 min; moreover, kinetic data can also be used to specifically detect an antibody. The specificity is determined predominately by the chosen immobilization techniques and is therefore equivalent for ELISA and quartz crystal microbalance measurements. However, as yet the obtained sensitivity of piezoelectric immunosensors is lower than that of an ELISA assay.

3.6. Detection of Cellular Systems

The application of microgravimetric acoustic sensors for the detection and characterization of pro- and eukaryotic cells has led to a number of interesting experimental findings as a result of the abundant information provided by such an analysis. Complex and time-consuming methods of cell biology may one day be replaced by faster, more highly resolving, and simpler techniques using TSM resonators as piezoelectric sensors. These sensors are particularly important in the food industry for the routine determination of bacterial cell numbers in diets, but it is also desirable in clinical areas to be able to determine cell numbers in body fluids online. Table 5 gives an overview of bacteria whose cell numbers were determined using piezosensors.

Most piezosensors used for the detection of bacteria in solution are based on antigen–antibody reactions in which the bacterium binds to the corresponding surface-confined antibody and thus can be monitored. In this way, the highest

Table 5. Summary of cell types that were investigated using quartz resonators. The lowest detection limit as well as the surface reaction that was used for detecting the cells are given.^[186]

Cell species	Cell number ^[187]	Surface reaction
<i>Staphylococcus epidermidis</i> ^[187]	$1 \times 10^2 - 4 \times 10^7$ cells mL^{-1}	liquidation of gelatin
<i>Listeria monocytogenes</i> ^[131]	$2.5 \times 10^5 - 2.5 \times 10^7$ cells per quartz	antigen–antibody
<i>Chlamydia trachomatis</i> ^[129]	$0.26 - 7.8 \mu\text{g mL}^{-1}$	antigen–antibody
<i>Vibrio cholerae</i> ^[122]	$> 10^5$ cells mL^{-1}	antigen–antibody
<i>Candida albicans</i> ^[188]	$10^6 - 5 \times 10^8$ cells mL^{-1}	antigen–antibody
<i>Enterobacteriaceae</i> ^[189]	$10^6 - 10^9$ cells mL^{-1}	antigen–antibody
<i>Salmonella typhimurium</i> ^[190]	$10^5 - 10^9$ cells mL^{-1}	antigen–antibody
<i>Salmonella typhimurium</i> ^[132]	$9.9 \times 10^5 - 1.8 \times 10^8$ CFU mL^{-1} ^[a]	antigen–antibody
<i>Salmonella typhimurium</i> ^[191]	$3.6 \times 10^6 - 102 \times 10^6$ cells mL^{-1}	cell growing on SS-agar ^[b]
<i>Pseudomonas cepacia</i> ^[192]	$> 3 \times 10^5$ cells cm^{-2}	cell growing on gold
<i>Staphylococcus epidermidis</i> ^[193]	3.9×10^{-2} % (v/v)	antigen–antibody, agglutination in solution
<i>Pseudomonas aeruginosa</i> ^[130]	$10^5 - 10^8$ cells mL^{-1}	antigen–antibody

[a] CFU = colony-forming unit (number of cells in a culture that can build a new colony in a layer). [b] SS = *Salmonella shigella*.

specificity is ensured. However, problems similar to those of immunosensors occur, such as nonspecific adsorption, which can only be abolished by blocking the free binding sites prior to the binding assay. In most cases a linear correlation between the bacterial cell number and frequency shift can be found, thus enabling one to calibrate the system for bacterial cell numbers. These studies are based on the assumption that the frequency shift upon cell binding can be attributed to a simple mass change as described by the Sauerbrey Equation. However, in 1993 Gryte et al.^[154] and Redepenning et al.^[155] were the first to point out that cells on quartz resonators in a medium cannot be treated like an ideal rigid mass. Cells are more likely to be presented as a viscous load similar to a fluid. A single cell layer on a 5 MHz quartz would give rise to a theoretical frequency shift of 5600 Hz according to the Sauerbrey Equation.^[155] The actual values are in general at least one order of magnitude lower than that. This observation is attributed to the concept that a cell body behaves like a viscoelastic body (for example, Voight or Kelvin body)^[156] under shear stress, which leads to the fact that besides an increase in the kinetic energy as a result of a mass change (increase in inductance L) an energy dissipation (increase in R) also occurs. It was shown that the shear wave within the system composed of the cell, extracellular matrix, and a water layer vanishes. This result was confirmed by experimental results in which cell multilayers and the adsorption of silica beads do not cause a detectable frequency shift.^[157]

In order to draw conclusions from the cell parameters assuming that they behave as viscoelastic bodies, an impedance analysis in the range of the resonance frequencies of the quartz is necessary. Complex shear moduli can be obtained by fitting electromechanical models to the obtained data. In Figure 25 the impedance behavior of a 5 MHz quartz in the presence and absence of adherent Madin darbne canine kidney (MDCK II) cells is shown. The quartz is highly damped because of the attached cells—an indication that they do not behave like a rigid mass. Modeling the cells attached to the resonator as a Newtonian liquid turns out to be too simplified to account for its impedance behavior on the resonator. In fact it is apparent from the spectra that an elastic contribution has to be considered.^[158] A detailed analysis of the individual components of an adherent cell monolayer on a quartz plate illuminates the complexity of the system. Besides

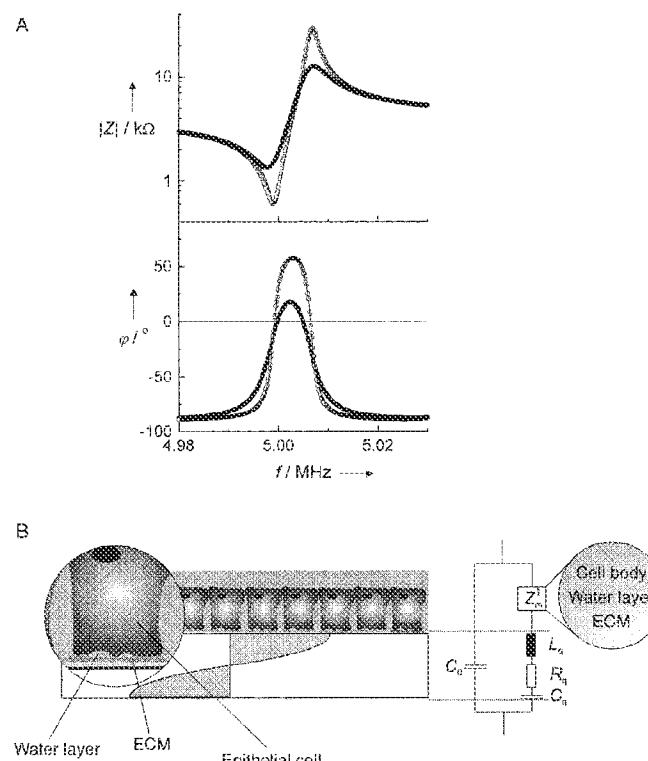


Figure 25. A) Influence of a confluent MDCK II cell monolayer on the impedance spectrum of a 5 MHz quartz resonator ($|Z|$ and φ); the spectra before (●) and after (○) scraping the cell layer from the surface are shown. The solid lines are the results of curve fitting using the BVD-equivalent circuit. B) Equivalent circuit of an adherent cell monolayer including extracellular matrix (ECM) and water layer. Z_m^1 represents the combined viscoelastic properties of adherent cells as well as the ECM and an adjacent water layer between the cell and the surface.^[167]

the actual cell layer, the extracellular matrix (ECM), a water film between the ECM and cell, and the medium on top of the cells need to be considered. It was shown that the influence of the extracellular matrix plays a pivotal role in the shear oscillation, while the mechanical properties of a cell-covered quartz resonator remain unaffected by the supernatant medium. The contribution of the ECM is mainly inductive and can therefore be considered as a simple mass added to the

resonator. Energy dissipation, however, could not be observed.^[159]

Damping of the shear oscillation by the presence of a confluent cell monolayer is dependent on the particular cell species and their ability to adhere on the surface. Loosely attached bovine aortic cells have little effect on the motional resistance R , while strongly adhered MDCK cells damp the oscillation of the quartz considerably (Table 6). This difference can be explained by the presence of a variable, thin water layer between the cell and the surface. Diminishing the thickness of the water layer by applying hyperosmotic stress, in which the osmolyte does not penetrate the cell layer (saccharose), leads to a dramatically increased damping of the shear oscillation (Figure 26).

Table 6. Change of the motional resistance R and inductance L of different cell species. The parameters were obtained from the fitting of the BVD-equivalent circuit before and after mechanical removal of the confluent cell layer.^[159]

Cell species	ΔR [Ω]	ΔL [μH]
MDCK I	755 ± 36	6.7 ± 0.7
MDCK II	992 ± 36	10.5 ± 0.8
Plexus epithelia	804 ± 43	16 ± 1.5
BAEC	55 ± 12	3.1 ± 0.5
3T3-fibroblasts	277 ± 20	2.5 ± 0.5

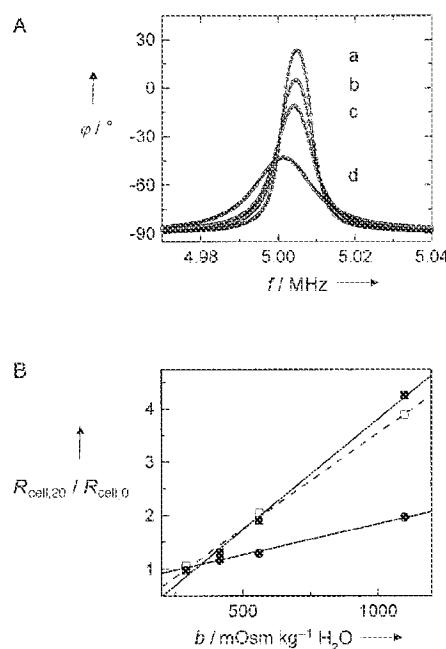


Figure 26. A) Phase spectra obtained from a quartz resonator covered with a confluent MDCK I cell layer upon sequential increase in the concentration of the osmotically active substances; a) 290, b) 415, c) 560, d) 1100 mOsm per Kg H₂O. The solid lines are the results of a fitting procedure using the BVD-equivalent circuit. B) Change of the relative motional resistance as a function of the molality of the osmotically active substances for MDCK I (□), MDCK II (■), and 3T3 cells (●). The motional resistance $R_{cell,20}$ was obtained 20 min after exchange of the isotonic medium for the indicated molality of the osmotically active substances, and is related to the initial resistance $R_{cell,0}$. The solid lines are the results of linear regressions.^[159]

Wegener et al.^[159] demonstrated that the observed increase in damping may probably not be attributed solely to a reduction of the cell volume;^[160] instead, an osmotic-driven water flow out of the cell–substrate interspace presumably occurs, and this moves the cells more closely to the quartz surface and increases the damping.^[161]

On the basis of the previous considerations, a quantitative analysis of the impedance data should ascribe a cell-covered quartz surface as a three layer model consisting of a rigid mass (ECM), a thin liquid layer between the ECM and cell body, and a semi-infinite viscoelastic body, the cell. A detailed analysis of this multicomponent system is the subject of current investigations.

Besides from data of confluent cell monolayers obtained under static conditions, time-resolved processes, such as cell adhesion or the influence of pharmacological substances, can also be monitored using the quartz-crystal microbalance in the active or passive mode. The process of cell adhesion is rather complex and characterized by several distinct processes which influence the oscillation behavior of the quartz resonator. Starting with the first physical contact of the cell with the surface, processes such as cell spreading, that is, the enlargement of the contact area of the cell with the surface, modification of the adhesion properties, excretion of extracellular matrix components, and changes of the cell's cytoskeleton influence the oscillation in various ways. A detailed study of cell adhesion based on a time-resolved frequency analysis was performed by Wegener et al.^[162] using different cell lines (MDCK I and II cells as well as 3T3-fibroblasts). Wegener et al. confirmed the presumptions of Redepenning et al.^[155] and Matsuda et al.^[163] that the frequency decrease is correlated with the number of cell–substrate contacts. Cells that do not form contacts with the surface as a result of the presence of contact-inhibiting peptides (RGD sequences) or cell death that prevent adhesion, do not change the resonance frequency of the quartz (Figure 27).

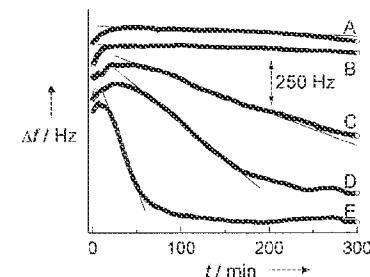


Figure 27. Time course of the resonance frequency during the adhesion of MDCK II cells starting with seeding densities of $(8 \pm 1) \times 10^5$ cm⁻² in the presence of A) 1 mM GRGDS; B) 1 mM RGDS; C) 2 mM RGD; D) 1 mM RGD, and E) 1 mM SDGRG. The solid lines are linear regressions to enable the apparent adhesion rates to be determined.^[162]

If a comprehensive impedance analysis of the quartz oscillation is not possible, or a higher time-resolution than provided by impedance analysis is required, changes in the dissipation factor $D = Q^{-1}$ of the oscillation, which represents the energy dissipation upon the binding of cells to the resonator surface, can be monitored. In this way Kasemo and

co-workers were able to detect the adsorption of monkey kidney epithelial (MKE), Chinese hamster ovary (CHO) cells, and neutrophiles by measuring the frequency shift of the quartz and the time-resolved energy dissipation simultaneously.^[164, 165] Otto et al.^[166] used the same technique to investigate the interaction of *E. coli* with surfaces varying in their hydrophobic behavior. These experiments showed that contact area, surface energy, ionic strength, and the cell surface influence the frequency and dissipation changes considerably.

If cell monolayers exhibit interesting barrier-forming properties as a consequence of the presence of tight junctions, the quartz-crystal microbalance can be combined with common electrochemical techniques. Such a setup allows the performance of a so-called quasi-simultaneous double-mode impedance analysis, as described by Janshoff et al.^[167] (Figure 28).

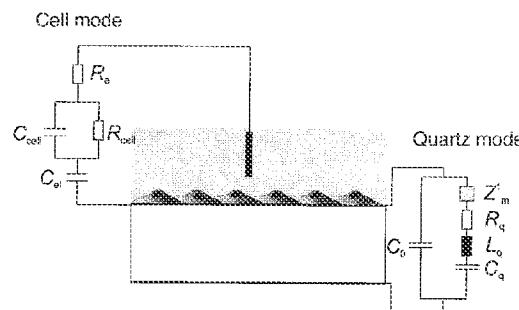


Figure 28. Schematic drawing of cells that are adhered to a quartz plate and the corresponding equivalent circuits used for the impedance spectra obtained in quartz mode and cell mode.^[167]

With this setup it is possible to detect electrical and viscoelastic properties of cell monolayers with one device. A cell monolayer can be described by a parallel RC element in series with a capacitance C_{el} , which represents the electrode–solution interface; the corresponding equivalent circuit is depicted in Figure 28. Figure 29 A shows the time course of the transepithelial and motional resistance after the seeding of MDCK II cells on a quartz resonator. Damping of the shear oscillation occurs immediately after seeding, whereas the transepithelial resistance can only be detected after 15 h. The adhesion of cells, which predominately determines the damping behavior of the cells, occurs long before the formation of tight junctions. Morphological changes of the cells induced by changes of the cytoskeleton can be monitored time-resolved with this device, as demonstrated by the example of cytochalasin D (alkaloid), which inhibits the polymerization of actin filaments (Figure 29B, C). The induced rounding of the cells by the degradation of the actin filaments results in a decreased contact area of the cells with the resonator and an increase in the average distance of the cells to the substrate; as a consequence a decrease in the characteristic damping of the shear oscillation by a confluent cell monolayer occurs. Moreover, it was shown that the transepithelial resistance collapses as a result of the large morphological changes.

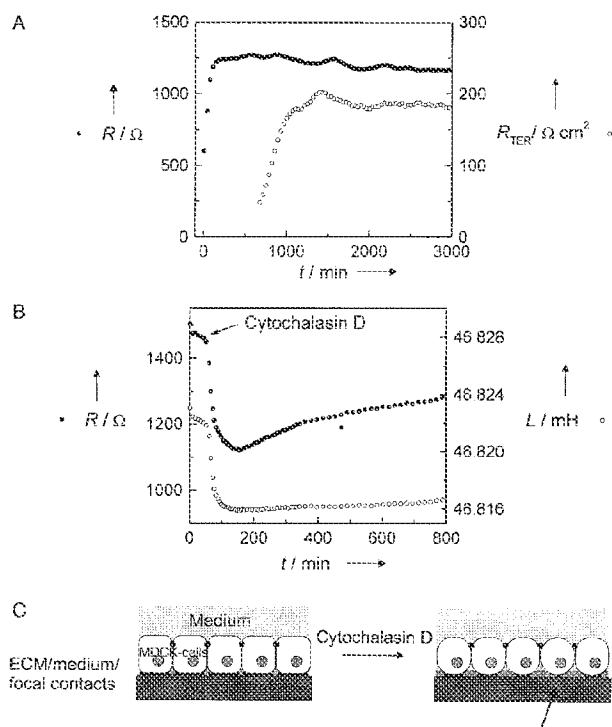


Figure 29. A) Time course of the motional resistance R (●) and transepithelial resistance R_{TER} (○) when a confluent MDCK II cell layer is formed on a 5 MHz quartz. The seeding density was $(1.5 \pm 0.2) \times 10^6$ cells per cm^2 . B) Time course of the motional resistance R (●) and the induction L (○) of a MDCK II-covered 5 MHz quartz upon addition of the alkaloid cytochalasin D with a concentration of $5 \mu\text{m}$. C) Conceivable scenario of the influence of cytochalasin D on a confluent cell layer. The arrow indicates the decreased contact area of the cells with the surface as a result of the rounding of the cell bodies.^[169]

3.7 Functionalized Vesicles as Model Systems for Cell Adhesion

As a consequence of the complexity of the cell and its contacts to the surface, it is quite difficult to address the question as to which properties of the cell mostly influence the resonance frequency shift. A simplified system to model the surface adhesion of cells in detail are receptor-doped lipid vesicles, which can interact with surface-confined ligands.^[168] Pignataro et al.^[169] scrutinized the interaction of biotinylated lipid vesicles with a streptavidin and avidin matrix. It turned out that the frequency decrease of the quartz was determined by the extent of biotinylation within the vesicles (Figure 30).

However, the frequency decrease could not solely be explained with the number of adsorbed vesicles on the surface. A complementary scanning force microscopy study demonstrated that an increase in the degree of biotinylation of the vesicles, that is, an increased number of biotin–streptavidin bonds, leads to a more pronounced flattening of the vesicles up to a biotin content where the vesicles disrupt and start to spread on the surface and form planar lipid bilayers. The authors drew the conclusion that the number of contacts influences the frequency response of the quartz. The connection between the contact area and frequency decrease was

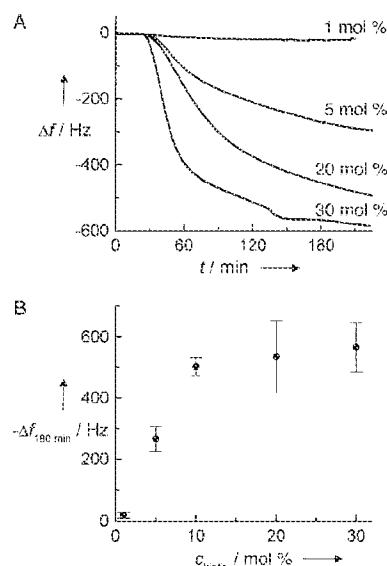


Figure 30. A) Frequency decrease of a 5 MHz quartz upon binding of vesicles doped with different amounts of biotin-X-DHPE to an avidin layer. B) Frequency shift 180 min after addition of vesicles.^[169]

also observed by Liebau et al.^[170, 171] They demonstrated that polymerized liposomes produce a significantly smaller frequency decrease than nonpolymerized ones. They explained this observation through a suppressed membrane fusion. As already demonstrated by Ohlson et al.^[172] and Janshoff et al.^[107] fusion of vesicles on the surface results in large frequency shifts of more than 230 Hz using a 5 MHz quartz. Presumably, a significant change in the surface energy might be responsible for the observed frequency shift.^[173] A more detailed explanation remains to be elucidated.

4. Development of Biosensors Based on Optical Transducers

In this section the acoustic resonators described above are compared with common state of the art optical transducers as used for bioanalytic purposes. In principle, information about the quantity of an analyte can be obtained from the characteristic properties of light, such as frequency, amplitude, phase, or polarization. In the area of biosensor development several label-free optical methods, such as surface plasmon resonance spectroscopy (SPR),^[174–176] grating coupler, reflectance interference spectroscopy (RIfs),^[177] and ellipsometry have been established.^[178] These techniques all detect the refractive index n and the film thickness d or the effective optical thickness (nd). The general principles of different optical techniques are depicted in Figure 31.

The specificity of those sensors is achieved by surface-immobilized receptive layers. The process of functionalization has to be adapted to the properties of the sensor surface. Thus, thiol chemistry is suitable for SPR and ellipsometry while silane chemistry is advantageous for glass surfaces (grating coupler, interferometry). In the following sections different techniques with significant commercial impact, such as SPR,

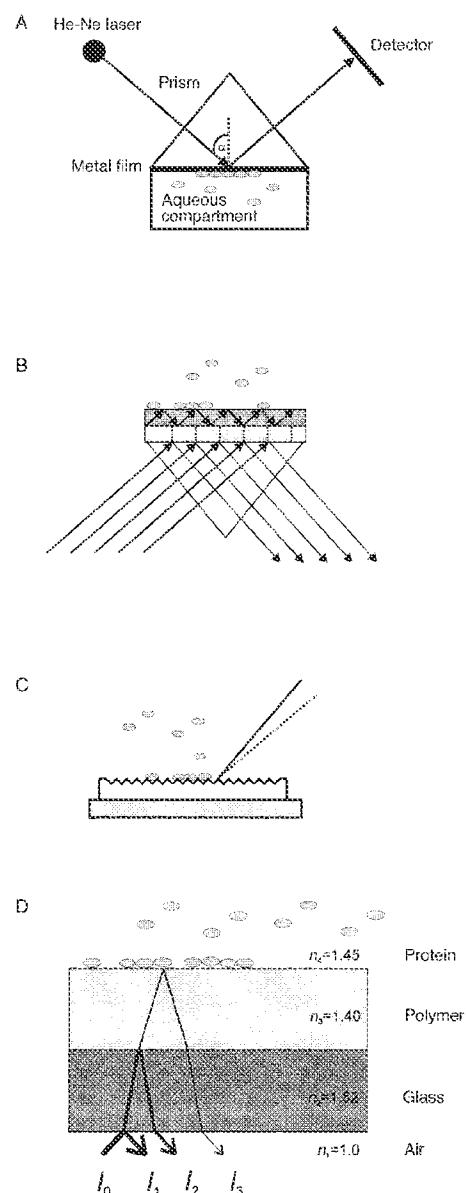


Figure 31. Schematic representation of the device principles of different optical label-free techniques. A) Surface plasmon resonance spectroscopy (SPR); B) resonance mirror (RM); C) grating coupler, and d) reflectometric interferometric spectroscopy (RIfs).

grating couplers, the resonance mirror technique, and interferometry (RIfs), are explained in more detail and different parameters, such as sensitivity, detection range, and their limitations, are discussed and compared to acoustic resonators.

4.1. Grating Coupler and Resonance Mirror Technique

Evanescence field techniques are based on multiple reflections at the interface between a substrate and a thin film ($d < 1 \mu\text{m}$) immobilized on a surface composed of biological receptive layers that show a lower refractive index than the

supporting substrate. The detection limit is considerably lowered when the system is equipped with an optical waveguide with multiple reflections since the relative propagation velocity of the guided wave depends not only on the refractive index of the medium but also on the number of reflections. Guidance of the wave is accomplished by an imprinted grating structure on the surface using a certain angle of incidence. Observing the intensity after the wave has traveled through the material at the end of the film is the easiest way of measurement.^[177]

However, in most cases this technique is used as a differential interferometer in which the different propagation velocities of the waves for different polarizations are considered. As *E*- and *B*-field vectors of the electromagnetic radiation are orthogonal, they will propagate with different velocity, dependent on the refractive index of the surface confined compound. The detectable phase difference, or strictly speaking its change, is a measure of the altered refractive index, for example, on the binding of an analyte to a receptive surface.

The resonance mirror (RM) technique^[177] is based on a prism coupler; the coupling conditions for two orthogonal waves of linear polarized light are met at different angles of incidence. The polarized waves experience a phase shift in the waveguide. Linear polarized light with an incident angle of 45° is used in a typical experiment. The observed ellipticity after travelling through the waveguide depends not only on the phase shift, and therefore considerably on the refractive index, but also on the adsorption layer at the interface. Thus, the ellipticity is again a direct measure of the amount of surface-bound analytes. The main applications of both methods in the field of biosensors are the detection of antigen–antibody reactions.

4.2. Surface Plasmon Resonance

Surface plasmon resonance spectroscopy (SPR) utilizes the evanescent field in the same fashion as the resonance mirror technique for the determination of the refractive index close to the sensor surface. Surface plasmons are longitudinal electron-density oscillations at the interface of a metal and a dielectric medium, for example. Surface plasmon resonance occurs by optical excitation only if the wave of the incoming light interacts with the free electrons of the metal and if the energy and momentum of the incident light beam correspond to those of the surface plasmons. The dispersion relation between frequency ω and wave number k of the plasmons at the interface [Eq. (18) ϵ_m and ϵ_d are the dielectric constants of the metal film and the dielectric medium, respectively] is decisive for excitation.

$$k = \frac{\omega}{c} \sqrt{\frac{\epsilon_d \epsilon_m}{\epsilon_d + \epsilon_m}} \quad (18)$$

Excitation is accomplished by attenuated total reflection, with the compound under investigation being immobilized on a glass prism covered with an evaporated metal film. In this setup, plasmons are excited by an evanescent electromagnetic field which induces oscillation of the free electrons in the

metal. Only p-polarized light is capable of exciting surface plasmons. However, these plasmons generate another electromagnetic field, which penetrates the dielectric medium closely attached to the metal surface. Only at a certain angle of incidence do the wave numbers of radiation and surface plasmons match, which leads to a resonance phenomenon in which the energy of photons is transferred to plasmons. Plasmon resonance can be observed as a sharp minimum in the reflectivity of the incident light beam. The angle of incidence of monochromatic light is varied during an experiment, and the reflectivity near the resonance monitored. As the wavelength is constant, the angle of the resonance minimum only depends on the dielectric constant of the medium covering the metal layer and is thus a measure of the concentration of an analyte adsorbed on the functionalized metal surface.

SPR can be used as a spectroscopic as well as a microscopic technique, which leads to an increasing number of applications of this optical method. Thus, SPR is used not only for studying classical ligand–receptor interactions in biochemistry but also for the detection of binding events at lipid membranes, similar to the quartz crystal microbalance technique. Special methods are required to modify these surfaces, which are essentially based on the fusion of liposomes onto hydrophobic surfaces. The interested reader is referred to the review articles of Salamon et al.^[175, 176] The high sensitivity at a distance up to 100 nm away from the gold surface allows the imaging of focal contacts of adherent cells by SPR microscopy.^[180]

4.3. Reflectometric Interference Spectroscopy

While both label-free methods discussed above detect changes in the refractive index upon analyte binding using an evanescent field, the reflectometric interference spectroscopy directly determines the effective thickness and thus the surface concentration of the analyte. This bound analyte layer exhibits a thickness of up to 10 nm in the case of biomolecules, but only 0.1 nm for low molecular weight compounds.^[177] The physical principle of this method is a wavelength modulation of the reflectivity of a thin transparent film (Figure 31D). When a film is illuminated with white light through a substrate it is reflected at both interfaces of the film. If monochromatic light is used, either constructive or destructive interference is observed, dependent on the phase shift; the interference obtained using white light varies upon the wavelength, so that a periodic modulation of the reflecting light intensity—an interference spectrum—results. Thus, the positions of the maxima and minima only depend on the film thickness at a given refractive index and angle of incidence. The constructive interference obtained by illumination along an axis coincident with the surface normal is given by Equation (19), in which m is the order of the maxima.

$$2nd = m\lambda \quad (19)$$

An increase in the thickness of the transparent layer results in a shift of the spectrum to larger wavelengths and is strictly valid only for transparent films with a thickness in the range of

the wavelength of the incoming light. This is not true for high molecular weight biomolecules and low molecular weight analytes. Nevertheless, a simple experimental trick enables one to obtain defined interference spectra, for example, a glass substrate is covered with a thick interference layer of a polymer material such as gels or oxides (SiO_2). This layer, which exhibits an interference pattern, is then functionalized by receptor molecules. As a consequence, a change in the thickness because of the adsorption of the complementary binding partner can be measured. The thickness change is far below the wavelength of radiation. This method allows measurements of thickness changes down to 1 pm resolution in effective optical thickness. Gauglitz and co-workers used this technique to quantify antigen–antibody reactions as an example of high molecular weight analytes as well as the binding of pesticides (triazine) as an example for low molecular weight analytes. They immobilized anti-pesticide antibodies on an interference layer and determined the thickness change, which was partially below 1 pm.

In special cases porous surfaces obtained from *p*-type silicon are used as an alternative to polymer films.^[181] These thin meso- to macroporous films serve not only for the generation of interference maxima and minima but also provide a signal enhancement arising from the large increase in the surface area within the porous matrix. The inner cavities of the pores need to be properly functionalized, which is achieved either by using trialkoxysilanes or Lewis acids and Grignard reagents to generate Si–C bonds.^[182–184] Reflectometry as well as the other optical methods can be applied to all kinds of binding problems and is an attractive alternative to common ELISA techniques, although it is less sensitive. Furthermore, reflectometry is suited to deal with thermodynamic and kinetic problems. Optical transducers as used in reflectometry reveal a high potential for detecting pharmaceutically relevant analytes.

5. Comparison of the Application of the QCM with Optical Methods

A detailed comparison of the QCM and SPR method was published in 1995 by Kößlinger et al.^[185] In this work a QCM and SPR spectrometer were realized in one setup. The authors

claimed that no significant difference between both techniques could be notified in terms of sensitivity and cross reactivity. Both measurement devices are equally suited for the online detection of chemical and biological analytes and work without chemical modifications of the analyte. The essentially different measurement principles (mass density for QCM and dielectric constant for SPR), however, lead inevitably to different limitations with respect to sensitivity, signal to noise ratio, accuracy, reproducibility, regeneration of the sensor surface, suitability for different complex analyte systems, handling, and cost.

For SPR transducers, the metal film is evaporated on glass substrates, which are transparent for lasers in the visible region, or silicon chips, which are transparent in the IR region.^[174] Optical components such as simple glass fibers, ATR-prism couplers, grating couplers, or integrated layer systems can be used as optical waveguides. The cost of such a setup is about two times higher than that of a QCM, which comprises a frequency counter, voltage supply, and an oscillator circuit. Table 7 summarizes typical properties of the different measuring devices.

The comparison of Kößlinger et al.^[185] basically demonstrates that both techniques are equally suited for bioanalytics. Affinity measurements can be satisfactorily performed with both devices (Figure 32).

The great advantage of SPR over QCM is its small sensor area ($5 \times 10^{-3} \text{ mm}^2$ in comparison to 5 mm^2 for QCM) and the higher sensitivity, namely, a greater number of particles is detectable on the effective sensor surface (10^{-17} mol for SPR and 10^{-14} mol for QCM). The quartz-crystal microbalance is, however, more suited for the determination of material properties such as the viscoelasticity of polymer films.

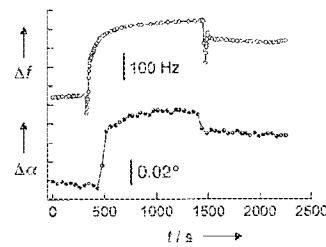


Figure 32. Change in the resonance frequency (○) and SPR angle (●) upon nonspecific adsorption of bovine serum albumin to a gold surface.^[185]

Table 7. Comparison of surface plasmon resonance spectroscopy (SPR) with the quartz-crystal microbalance (QCM; 20 MHz quartz) on the basis of their special properties.^[185]

	QCM	SPR
wave equation	Christoffel equation	Maxwell equation
wave type	elastic shear waves	surface plasmons
basic physical quantities	mechanical impedance Z	reflectivity
measuring quantities	frequency, quality factor, impedance	angle of reflectivity minimum
material parameter	film thickness, density, viscosity, surface tension, viscoelasticity, ionic strength	dielectric constant, film thickness
penetration depth in water	126 nm	150 nm ($\lambda = 1300 \text{ nm}$)
influence of ionic strength	can be neglected	large
sensitive area	5 mm^2	0.005 mm^2
detection limit	10^{-14} mol	10^{-17} mol
thickness sensitivity/protein film	184 Hz nm^{-1}	$0.0263^\circ \text{ nm}^{-1}$
minimal significant change of the measuring quantity	20–25 Hz	0.005°
minimal detectable mass	30 ng per 5 mm^2	0.5–5 ng per $5 \times 10^{-3} \text{ mm}^2$
sensitivity calibration	electrolysis	–

6. Summary and Outlook

In the last few years the quartz-crystal microbalance has developed from a pure mass sensor in the gas phase into a versatile tool in chemo- and bioanalytics which not only provides information about binding events at surfaces, but also reveals material-specific quantities, such as elasticity moduli, surface charge densities, and viscosity. Indirectly, one can also deduce conformational changes of proteins as well as the water content and net charge of biomolecules from the data. Potential applications are quantification of cell adhesion and the determination of viscoelastic properties of adherent cells. It is expected that TSM resonators will become to be a real alternative to conventional cell-specific techniques, such as cell counting and optical microscopy, to control cell culture. Many active substances directly influence cell adhesion. Hence, the quartz-crystal microbalance is a fast and sensitive device providing information about cell–substrate interactions, the alteration of the number of focal contacts, or the distance of the cell from the substrate. Moreover, the possibility of combining the quartz-crystal microbalance with other techniques, for example, optical (SPR) or electro-mechanical techniques (EQCM), will increase the knowledge about processes at interfaces in biochemistry as they occur at cell surfaces. In this way double mode impedance analysis allowed alterations of the barrier properties and cell substrate interactions of cell monolayers to be correlated in a time-resolved fashion and quasi-simultaneously. While the electrochemical QCM is already routinely used in electrochemistry for monitoring deposition and corrosion processes, a combination of voltammetry, chronoamperometry, and impedance analysis with QCM is rarely used in bioanalytics.

In summary, the detection limit of the quartz-crystal microbalance is poorer than that of optical methods such as SPR or RI_{FS}, which is partially a result of the larger sensor surface area, and partially a result of the inherent sensitivity. In most cases, a lower sensitivity does not hinder the investigation of biological problems since equilibrium thermodynamic and kinetic data cannot be obtained beyond picomolar concentrations as a consequence of the limited volume. A continuous depletion in solution would result—this is also true for surface plasmon resonance spectroscopy, though SPR spectroscopy needs a considerably smaller detection area, since the sensor area covered with adsorbed molecules is in general larger than the detected one. As far as the QCM is concerned miniaturization is naturally limited, which plays an important role with respect to the desired automation because of the need for small reaction chambers. The power of piezoelectric sensors is definitely the vast quantity of available information that can be obtained and the reliable determination of thermodynamic and kinetic data. The method allows the use of almost any material from metals to dielectric surfaces. In order to achieve lower detection limits for minor component analysis it is necessary to use piezoelectric sensors with higher mass sensitivity, such as APM and FPW sensors, though these methods need to be further developed.

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- [13] In addition, in the case of small resonators with large thickness, the resonance frequency depends considerably on the lateral dimensions of the circular-shaped plate as apparent from the second term.
- [14] An AT-cut quartz crystal exhibits thickness excitation (TE) and lateral field excitation (LFE); the first one generates transversal the last one longitudinal waves.
- [15] Notably, Equation (2) describes basically the serial resonance ($Z \rightarrow 0$). The difference between the antiresonance ($Z \rightarrow \infty$) and serial resonance is manifested in the electroacoustic constant k_1 : $\omega_a^2 - \omega_s^2 = 8k_1(\bar{c}_{66}/\rho_q d_q^2)$.
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Comparison of surface plasmon resonance spectroscopy and quartz crystal microbalance techniques for studying DNA assembly and hybridization

Xiaodi Su^{a,*¹}, Ying-Ju Wu^{a,1}, Wolfgang Knoll^{a,b}

^a Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602, Singapore

^b Max-Planck-Institut für Polymerforschung, Ackermannweg 10, 55128 Mainz, Germany

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Abstract

In this study we evaluate the strengths and weaknesses of surface plasmon resonance (SPR) spectroscopy and quartz crystal microbalance (QCM) technique for studying DNA assembly and hybridization reactions. Specifically, we apply in parallel an SPR instrument and a 5 MHz QCM device with dissipation monitoring (QCM-D) to monitor the assembly of biotinylated DNA (biotin-DNA) on a streptavidin-modified surface and the subsequent target DNA hybridization. Through the parallel measurements, we demonstrate that SPR is more suitable for quantitative analysis of DNA binding amount, which is essential for interfacial DNA probe density control and for the analysis of its effect on hybridization efficiency and kinetics. Although the QCM is not quantitative to the same extent as SPR (QCM measures the total mass of the bound DNA molecules together with the associated water), the dissipation factor of the QCM provides a qualitative measure of the viscoelastic properties of DNA films and the conformation of the bound DNA molecules. The complexity in mass measurement does not impair QCM's potential for a kinetic evaluation of the hybridization processes. For quantification of target DNA, the biotin-DNA modified SPR and QCM sensors are exposed to target DNA with increasing concentration. The plots of SPR/QCM signals versus target DNA concentration show that water entrapment between DNA strands make the QCM sensitivity for the hybridization assay well comparable with that of the SPR, although the intrinsic mass sensitivity of the 5 MHz QCM is ~20 times lower.

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1. Introduction

Surface plasmon resonance (SPR) spectroscopy and the quartz crystal microbalance (QCM) technique have been known independently as surface analytical techniques capable of *in situ* monitoring of interfacial processes. One of the current trends in SPR and QCM research is to use a combined SPR and QCM data collection mode and analysis [via either dual probed devices that have both the QCM and SPR functions (Baily et al., 2002; Laschitsch et al., 2002; Bund et al., 2003; Wang et al., 2003; Zhou et al., 2004) or parallel

measurements using separate instruments (Graneli et al., 2004; Laricchia-Robbio and Revoltella, 2004; Larsson et al., 2003; Höök et al., 2001a)] in order to obtain complementary details of a particular binding event. This becomes possible as SPR spectroscopy and QCM are based on different physical principles; each method being sensitive to different properties of the materials studied. SPR spectroscopy, for example, is an optical technique that detects changes in the refractive index of thin films assembled on a noble-metal surface. The measured signals are proportional to the molecular weight of the adsorbed materials, and can be used to quantify the number density of different types of adsorption. On the other hand, QCM is an acoustic wave device. It measures thin films mechanically coupled to a metal electrode on a quartz disk. The QCM oscillation frequency and quality are

* Corresponding author. Tel.: +65 68748420; fax: +65 68720785.

E-mail address: xd-su@imre.a-star.edu.sg (X. Su).

¹ These authors contributed equally to this paper.

related to the mass loading and the viscoelastic properties of the adsorbed materials, respectively. For macromolecular films, QCM is sensitive to both the bound molecules and the associated solvent, e.g. water.

Since the earliest SPR- and QCM-based biosensors were reported in 1983 (Liedberg et al., 1983) and 1972 (Shons et al., 1972), respectively, these two devices have been widely used for biological analysis and clinical diagnosis (Cavic et al., 1999; Englebienne et al., 2003). The merits of the SPR- and QCM-based bioassays lie in the fact that the biomolecular binding reactions can be monitored in a label-free manner, which shortens the assay time and eliminates the use of hazardous materials and expensive lab equipments. In addition, the liquid cell configuration of the two devices makes them suitable for real-time studies of bioaffinity reactions at relevant solution conditions of temperature, flow rate, pH, ionic strength, etc.

Recently, increasing concern has been raised about the strengths and weaknesses of SPR and QCM devices if used as sensing platforms for various biological analyses. Comparisons of SPR and QCM performances for immunoassays (Kösslinger et al., 1995; Sellborn et al., 2003; Su and Zhang, 2004), blood plasma coagulation determination (Vikinge et al., 2000), enterotoxin detection (Spangler et al., 2001), enzymatic analysis (Su and O’Shea, 2001), structural analysis of proteins (Laricchia-Robbio and Revoltella, 2004; Stevens et al., 2004), DNA hybridization analysis (Larsson et al., 2003; Cho et al., 2004), and DNA–protein interactions (Su et al., 2005) have been reported. In these studies, correlations between results obtained using the two techniques are reported and SPR and QCM devices are evaluated to determine whether the sensitivity, reliability, and ease of operation are suitable for the specific bioassays.

In our study here, we compare the strengths and weaknesses of the SPR and QCM techniques for the study of DNA assembly and hybridization reactions. Specifically, we apply in parallel an SPR instrument (AutoLab ESPR) and a 5 MHz QCM device with dissipation monitoring (QCM-D) to follow the assembly of a biotinylated 30-mer oligonucleotide on a streptavidin-modified gold electrode employed for the hybridization analysis. By a combined data collection and analysis (SPR angle shift, QCM frequency shift, and QCM energy dissipation factor), we demonstrate how the different sensing principles of the SPR and QCM benefit the study of DNA film structure and how the DNA probe density affects hybridization efficiency/kinetics, as well as the viscoelastic properties of the DNA films. Also, we compare the QCM and SPR sensitivity for target DNA quantification.

2. Experimental

2.1. Materials and surface preparation

Streptavidin (SA) was purchased from Sigma. 30-Mer oligonucleotides were obtained from MWG (Germany).

The probe DNA was prepared with a biotin label at the 5'-end (5'-biotin-GCACCTGACTCTGTGGAGAAGTCT-GCCGT-3') and the target DNA contains fully complementary sequences to the probe DNA (3'-CGTGGACTGAGGACACCTTTCAGACGGCA-5'). Phosphate buffered saline (PBS), composed of 10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, pH 7.4, was used as a carrier buffer for SA immobilization, DNA assembly and target DNA hybridization.

The gold electrodes of the SPR and QCM disks were first cleaned with hot piranha solution (a 3:1 mixture of H₂SO₄ and H₂O₂. Cautions!). The freshly cleaned disks were then immersed in a binary biotin-containing thiol mixture (10% biotin–thiol, 90% ethylene glycol–thiol at a net concentration of 1 mM in ethanol) overnight (these thiol compounds were synthesized in our laboratory in Mainz). The formula can be seen in previous papers (Su et al., 2004; Spinke et al., 1993). After rinsing with ethanol followed by a drying step using nitrogen, the disks were ready to use.

2.2. SPR measurement and data treatment

SPR measurements were conducted using a double channel, AutoLab ESPR (Eco Chemie, The Netherlands). The configuration of this equipment is described elsewhere (Su and O’Shea, 2001). The instrument is equipped with a cuvette. Gold sensor disks (diameter 17 mm) mounted to the optical lens through index-matching oil form the base of the cuvette. An autosampler (Eco Chemie, The Netherlands) is used to inject or remove the tested solutions, but the measurement of the SPR angle shift ($\Delta\theta$) was done at non-flow liquid condition, i.e. with the circulating pump paused, and at room temperature. The noise level of the SPR angle is ~ 1 mdegree.

The measured SPR angle shifts were converted into mass uptakes using a sensitivity factor of 122 mdegrees = 100 ng/cm². In the data conversion we assumed the same equivalent SPR response per unit coverage for protein, single-stranded DNA and double-stranded DNA, respectively. This assumption is reasonable (Larsson et al., 2003; Peterson et al., 2002), as the dn/dC values (the incremental change in refractive index with concentration) for protein and DNA are very similar.

2.3. QCM-D measurement and data modeling

The QCM-D measurements were conducted using a Q-sense instrument (Q-Sense, Göteborg, Sweden). This instrument allows for a simultaneous measurement of frequency change (Δf) and energy dissipation (ΔD) change by periodically switching off the driving power of the oscillation of the sensor crystal and by recording the decay of the damped oscillation. The time constant of the decay is inversely proportional to D , and the period of the decaying signal gives f . Five megahertz AT-cut quartz crystals (Q-Sense AB, Göteborg,

Sweden) were used as the reaction carriers. The Sauerbrey sensitivity of these crystals is $1 \text{ Hz} = 17.7 \text{ ng/cm}^2$. The frequency and dissipation responses were recorded at around 15, 25 and 35 MHz, corresponding to the overtones, $n = 3, 5$ and 7, respectively. For better clarity, only the normalized frequency shift ($\Delta f_{\text{normalized}} = \Delta f_n/n$) and the dissipation shift, ΔD , for the 3rd overtone are presented. Data at other overtones are included for the modeling of the effective mass loading, effective thickness and shear viscosity using a Voigt-based representation (Höök et al., 2001a,b). During the measurements, the crystals were mounted in a liquid chamber, designed to provide a rapid, non-perturbing exchange of the liquid (non-flowing liquid) over one side of the sensor. The QCM measurements were conducted at room temperature, and the noise level of the frequency and dissipation factor with liquid load are $\sim 0.3 \text{ Hz}$ and $\sim 2 \times 10^{-7}$, respectively.

3. Results and discussion

3.1. Streptavidin and DNA assembly on gold

The multilayered surface architecture involved in this study is schematically illustrated in Fig. 1. Firstly, the gold surface is treated with the biotin-containing thiol mixture, forming an optimized matrix onto which a streptavidin monolayer is assembled via one or two biotin–streptavidin linkage(s) (Spinke et al., 1993). Biotinylated DNA (biotin-DNA) is then bound to the streptavidin surface via the remaining biotin-binding sites for capturing complementary target DNA through hybridization reactions. Fig. 2 shows the SPR response ($\Delta\theta$ versus time) and QCM response (Δf and ΔD versus time) of the surface binding reactions, starting from the exposure of the thiol treated surface to: (i) streptavidin (0.2 mg/mL), followed by (ii) biotin-DNA assembly (1 μM), and finally (iii) target DNA hybridization (1 μM). Using the intrinsic mass sensitivity of the AutoLab SPR instrument and the Sauerbrey sensitivity of a 5 MHz QCM, the measured SPR angle shifts and QCM frequency shifts are converted to mass uptakes in units of ng/cm^2 as shown in Fig. 3. Also included

in Fig. 3 is the QCM mass uptake obtained via a Voigt fitting of the QCM-D responses.

Considering the molecular weights of 60 kDa for SA, 9.6 kDa for the biotin-DNA, and 9.2 kDa for the target DNA, the measured SPR mass uptakes (SA 379.7 ng/cm^2 , biotin-DNA 92.3 ng/cm^2 , and target DNA 51.5 ng/cm^2) suggest a biotin-DNA/SA binding ratio of 1.5 and a hybridization efficiency of 58% (defined as the fraction of hybridized target coverage divided by the immobilized probe coverage at saturation). These results are in good agreement with what we obtained using a surface plasmon diffraction technique (Yu et al., 2004). It is not surprising that the two remaining biotin-binding sites on the immobilized SA molecules do not simply result in a biotin-DNA/SA ratio of 2. This is because the distance between the two biotin-binding sites on the well-ordered SA film is close to the hydrodynamic diameter of DNA. The combination of electrostatic repulsion and steric hindrance may prevent efficient coupling of two biotin-DNA molecules per streptavidin (Larsson et al., 2003; Höök et al., 2001a,b).

It is known that, unlike SPR, QCM is sensitive to the water associated with the macromolecules (Larsson et al., 2003; Höök et al., 2001a). For most macromolecular films, e.g., protein and DNA, the measured QCM mass uptake (via Δf) is always higher than the optical mass. The difference can be as high as a factor of 10, depending on the nature, the conformation of the adsorbed molecules, and the liquid medium used (Höök et al., 2001a; Cho et al., 2004). In our system where multiple layers of streptavidin and DNA films are involved, the difference between the QCM mass (Δm_{QCM}) and SPR mass (Δm_{SPR}) is 1.2, ~ 4 , and ~ 7 for the SA film, the single-stranded biotin-DNA film, and the captured target DNA, respectively (Fig. 3). In combination with the measured QCM damping parameter (ΔD in Fig. 2B), we found that, also in agreement with previous observations (Höök et al., 2002), as an overall trend that the higher the dissipation of the film (represented by a higher $\Delta D/\Delta f$ value) the larger is the difference between Δm_{QCM} and Δm_{SPR} .

For streptavidin films assembled on biotin-containing surfaces, previous studies have indicated that the protein

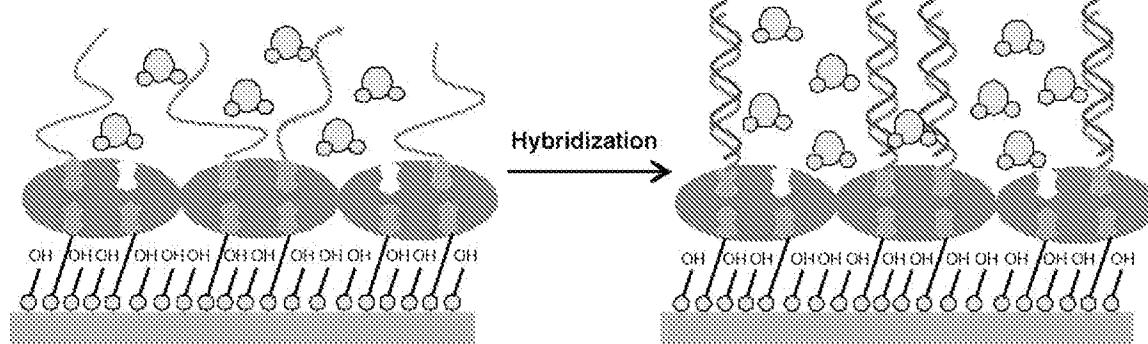


Fig. 1. Schematic illustration of the binding reactions involved in the study, including streptavidin immobilization on biotin–thiol treated gold surface and biotin-DNA assembly for target DNA hybridization. Also shown is the water entrapment between DNA strands.

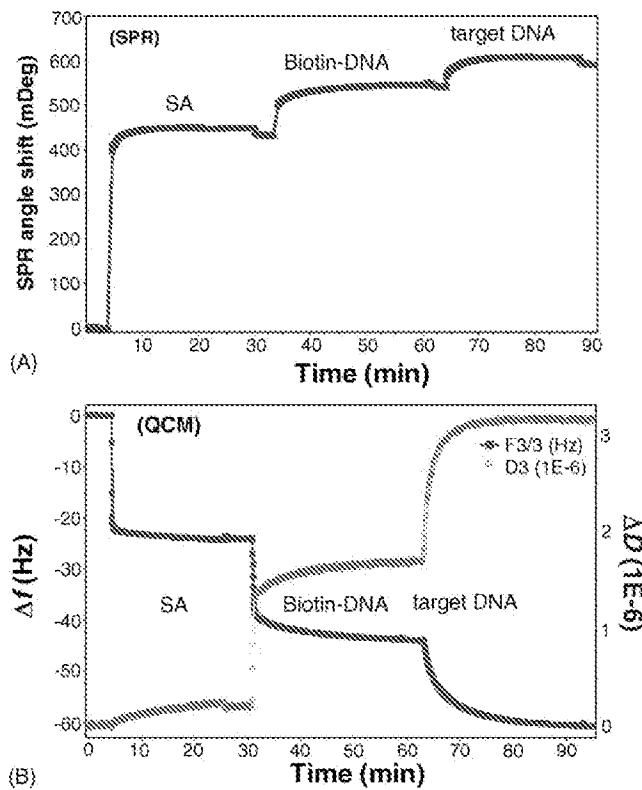


Fig. 2. (A) SPR and (B) QCM-D measurements of the surface binding reactions shown in Fig. 1, starting from the exposure of the thiol treated surface to (i) streptavidin (0.2 mg/mL in PBS), followed by (ii) biotin-DNA (1 μ M in PBS), and finally (iii) target DNA (1 μ M in PBS). At saturated binding after each exposure, the solutions were exchanged to pure buffer.

molecules are in a highly ordered 2D arrangement with two of the biotin-binding sites being occupied by the surface biotin residues, and the other two facing towards the solution (Knoll et al., 2000; Larsson et al., 2003; Höök et al., 2001b). In the QCM-D measurement of the SA film formed on the biotin–thiol treated surface in this study, the barely detectable ΔD increase ($<0.2 \times 10^{-6}$) induced by the mass loading ($\Delta f = 23.5 \pm 0.8$ Hz) indicates that the well-ordered SA film exhibits a highly rigid structure and the protein molecules can

fully couple with the shear motion of the crystal. For such a stiff film, the measured Δf contains mostly the mass effect with a negligible viscoelasticity contribution. This explains why the QCM mass (both the Sauerbrey mass and Voigt-mass) for the SA film is rather close to the SPR mass, the latter being better reflective of the molar mass of the protein film as it measures the refractive index changes if water is replaced by molecules.

For the biotin-DNA assembly and the successive target DNA hybridization, however, the mass accumulations (Δf decreases by 22.5 ± 1.0 and 18.5 ± 0.9 Hz, respectively) are accompanied by remarkable ΔD increases ($\Delta D = 1.4 \pm 0.1 \times 10^{-6}$ and $1.6 \pm 0.1 \times 10^{-6}$, respectively), indicating that the DNA films exhibit a highly dissipative structure. This supports a structural model in which the side-on attached biotin-DNA layer on the SA film is composed of elongated and flexible molecules primarily oriented outward from the surface. The larger than expected mass uptake obtained can be explained by taking into account the water molecules coupled between the DNA strands (see schematic illustration in Fig. 1). In other words, the DNA films are sensed by the QCM as viscoelastic brushes composed of DNA molecules and coupled solvent. Also noted from Fig. 3 is the observation that for the viscoelastic DNA films, the obtained QCM mass from the Voigt modeling is greater than the classical Sauerbrey mass by a factor of ~ 1.3 , contrary to the good agreement for the rigid SA film. This analysis emphasizes the importance of using a viscoelastic representation for the estimation of the coupled mass of viscoelastic films.

3.2. DNA film structure assayed by QCM-D

Although the QCM response is not quantitative in the same sense as is the SPR data, the analysis of the energy dissipation, ΔD , and/or its magnitude in relation to Δf , $\Delta D/\Delta f$, the induced energy dissipation per coupled unit mass, provides insights about the mechanical/structural properties and conformational changes of the adsorbed films (Larsson et al., 2003). In this study, to assess the viscoelasticity or conformational changes of the single-stranded biotin-DNA upon duplex formation, the $(\Delta D/\Delta f)_{\text{duplex}}$ value is calculated using the total mass uptake of the biotin-DNA plus target DNA and the total dissipation increase after hybridization. The obtained $(\Delta D/\Delta f)_{\text{duplex}}$ (72×10^{-9} Hz $^{-1}$) is greater than that of the single-stranded biotin-DNA (60×10^{-9} Hz $^{-1}$), indicating that the formed DNA duplex film is more dissipative or more flexible (detailed discussion on probe density effects is made in the next section). This explains why a much larger than expected QCM mass (~ 7 times of the SPR mass) is observed for the target DNA.

Through the Voigt modeling of the QCM-D data, we obtained the time evolution of the changes in the effective thickness of the DNA films (figure not shown). It is noted that the single-stranded, 30-mer biotin-DNA forms a layer of coiled oligonucleotides of 3.5 nm, which are stretched out to 6.9 nm during the duplex formation. This conformational change of

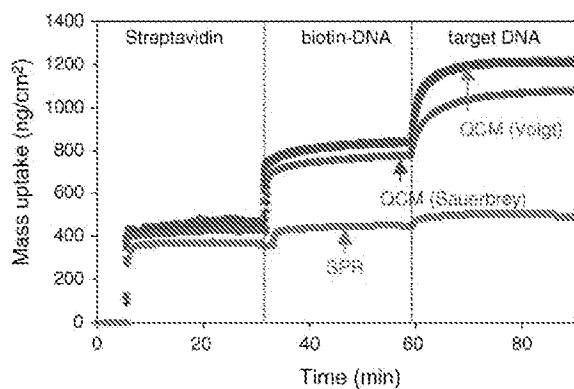


Fig. 3. Mass uptakes measured by the SPR and QCM-D. The Sauerbrey relationship and Voigt modeling are used for the calculation of QCM mass.

the overall DNA structure explains the increased $\Delta D/\Delta f$ ratio upon target hybridization, which is due to further water entrapment.

In a previous study, the QCM-D technique has been used for studying biotin-DNA assembly on streptavidin films constructed on biotin-doped lipid membranes (Larsson et al., 2003). The QCM-D behavior of the DNA films we observed in our study is not exactly the same as the similar 30-mer DNA in the reference system. We believe it is the supporting matrix (the thiol treated, rigid gold in our system, and the soft, mobile lipid membrane in the reference systems) mediates the DNA behavior. First of all, the assembly of the biotin-DNA on the soft, mobile lipid membrane matrix caused a Δf of ~ 21 Hz, being very similar to what we obtained ($\Delta f = 22.5$ Hz) for the 30-mer biotin-DNA on the thiol treated, rigid gold substrate in our system. However, the induced ΔD in the mobile reference system is much larger ($\Delta D = 2.8 \times 10^{-6}$) than that in our system ($\Delta D = 1.4 \times 10^{-6}$), leading to a $\Delta D/\Delta f$ ratio ($140 \times 10^{-9} \text{ Hz}^{-1}$) being much greater. This suggests that the formed biotin-DNA film on the soft, mobile membrane matrix exhibits a much higher flexibility, which would be understandable if taking into account the fluidic nature of the lipid membrane matrix. Secondly, upon hybridization the duplex DNA film in their system become less flexible ($\Delta D/\Delta f$ reduced to $121 \times 10^{-9} \text{ Hz}^{-1}$), contrary to the duplex DNA behavior in our system. Thirdly, the reference study reported a $\Delta D - \Delta f$ pattern with no significant coverage-induced phase change as we observed at the similar DNA concentration. With these discussions we intend to emphasize that the properties of the underlying supporting matrix has significant effects on the structure of the build-up molecular films. These effects are successfully detectable by the QCM-D measurements.

3.3. Probe density effects on hybridization efficiency, hybridization kinetics and DNA conformation

Previous studies indicate that for solid–liquid phase DNA hybridization, the density of the immobilized probe DNA has significant effects on hybridization efficiency and hybridization kinetics (Georgiadis et al., 2000; Peterson et al., 2001). In this study, biotin-DNA probe density control is achieved by varying the concentrations used for the assembly (from 50 nM to 1 μ M). The probe density effects on hybridization efficiency (HE%), hybridization kinetics, and the probe density-dependent viscoelasticity properties of the DNA films are studied through the SPR and QCM measurements of target DNA hybridization at a fix concentration of 1 μ M. Fig. 4A shows the SPR measurements of the biotin-DNA assembly at 100, 200 and 1000 nM, and the successive target hybridizations. The measured SPR angle shifts for the biotin-DNA at saturation are 50, 78 and 113 mdegrees, respectively, corresponding to probe densities of 2.6×10^{12} , 4.0×10^{12} , and 5.8×10^{12} molecules/cm², respectively. The successive hybridization signals are 38, 53, and 61 mdegrees, respectively, leading to HE% of 79, 71, and 56, respectively, taking into

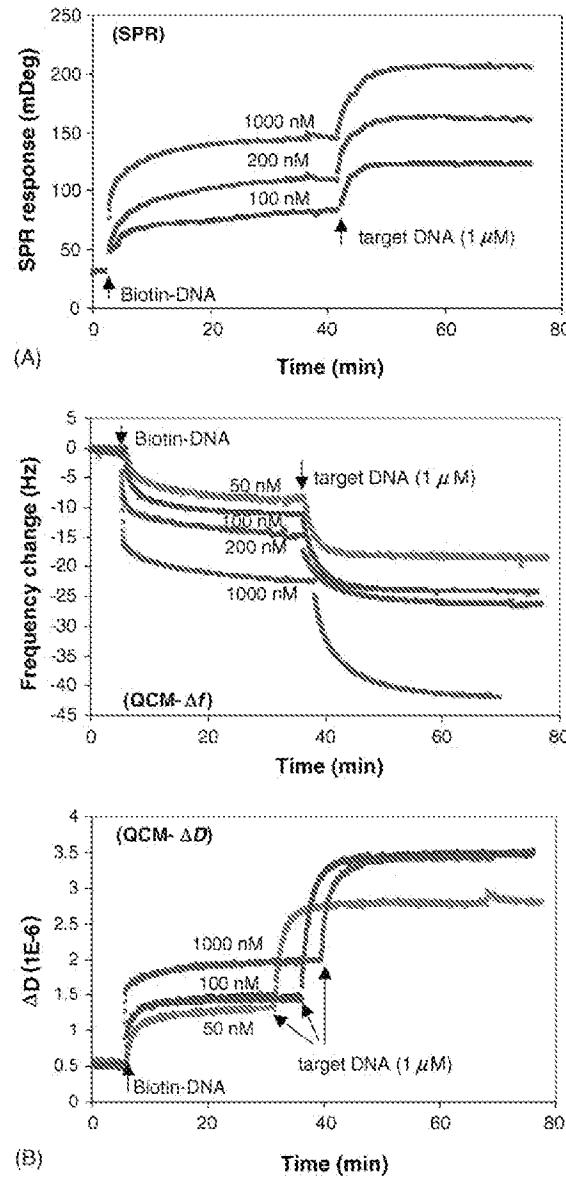


Fig. 4. (A) SPR and (B) QCM (Δf and ΔD) measurements of biotin-DNA assembly at varied concentrations and the successive target DNA hybridization at 1 μ M.

consideration the MW of the probe (9.6 kDa) and the target DNA (9.2 kDa). In a previous study, a similar HE% range of 80–40% was reported for a 25-mer SH-DNA system within a similar probe density range of 2.5×10^{12} molecules/cm² (Peterson et al., 2001). In general, the HE% decreases with the increase of the probe density, due to repulsive electrostatic and steric interactions.

The parallel QCM-D measurement of the probe density-dependent target hybridization is shown in Fig. 4B, displaying Δf and ΔD induced by the biotin-DNA assembled at 50, 100, 200, 1000 nM and the successive target hybridization at 1 μ M. The Δf values for the biotin-DNA/target DNA are 8.7/11.0, 10.5/13.1, 13.6/15.2, and 22.5/19.3 Hz, respectively (the correlation between biotin-DNA concentration and

ΔD response is given later). At first, these frequency values seem to correspond to ‘HE %’ of 126, 123, 110, and 83, respectively, if one does not take into account the viscoelastic contributions to the Δf values. These ‘HE%’ values are obviously impossible. The over estimated target DNA binding efficiencies can be attributed to the conformational change of the overall layer as discussed earlier: that is, the hybridization process induces a remarkable increase in the layer thickness and in the overall flexibility ($\Delta D/\Delta f$), meaning that upon hybridization the DNA molecules become more stretched-out and elongated. As a result, further entrapment of water within the formed DNA duplex is detectable by the QCM.

Although the analysis of HE% using frequency responses failed, the analysis of the time-dependent QCM response enables an evaluation of the hybridization kinetics as does SPR. Fig. 5A and B shows the SPR and QCM measurements, respectively, of the target hybridization kinetics as a function of probe density (data are from Fig. 4A and B, respectively, but are normalized). Results show that, also in agreement with previous studies (Peterson et al., 2001), at a lower probe density, the initial hybridization rate is relatively faster, reaching a maximum within 10–15 min. The slower hybridization rate at a higher probe density is most probably due to the repulsive electrostatic and steric interactions that increase with the increasing probe density. Apparently, the water association (or the complexity in the mass measurement using the QCM frequency) does not impair the qualitative analysis of the hybridization kinetics. This forms the basis of using QCM for a kinetic evaluation (Keller and Kasemo, 1998).

In addition to the effects on the target hybridization efficiency and kinetics, the probe density has strong influence on the viscoelastic properties of the DNA films as can be seen from the ΔD values (Fig. 4B, ΔD). Table 1 is a summary of the $\Delta D/\Delta f$ ratios measured for the biotin-DNA films at different density and the $\Delta D/\Delta f$ ratios of the corresponding DNA duplex layers. Also shown is the difference between the QCM and SPR mass for the biotin-DNA layer. At a lower probe density, the biotin-DNA films tend to be more dissipative (higher $\Delta D/\Delta f$), meaning that the films contain more water, as being revealed by the larger difference of the QCM and SPR mass. At each probe density, upon the formation of

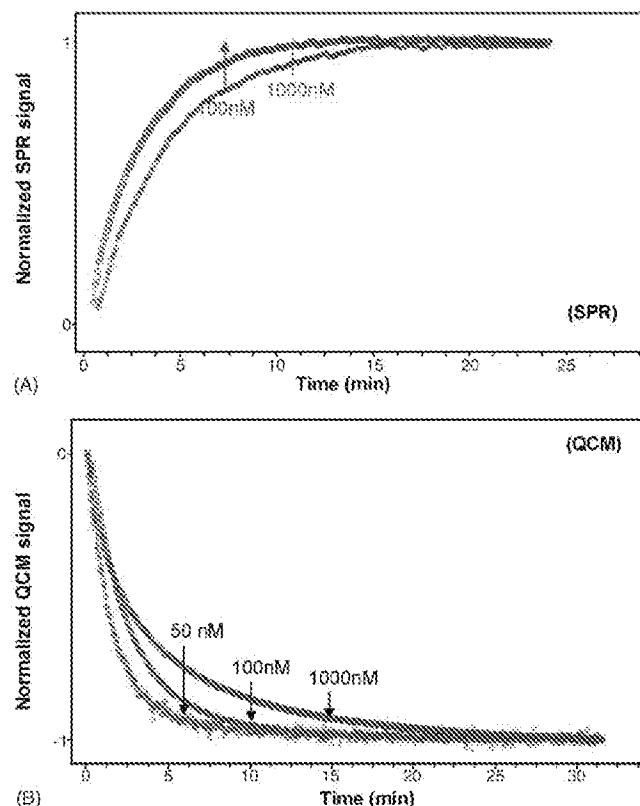


Fig. 5. (A) SPR and (B) QCM measurements of hybridization kinetics as a function of biotin-DNA probe density. The data are from Fig. 4, but are normalized.

the DNA duplex, the $\Delta D/\Delta f$ values increase, reflecting the increase of the flexibility of the duplex DNA films.

3.4. Quantification of target DNA

One of the major concerns about an SPR instrument and a QCM setup if used for DNA hybridization analysis is related to the quantification of the target DNA concentration. Through titrating the surface bound biotin-DNA (prepared at 1 μ M) with increasing the target concentration (10 nM to 1 μ M), we observed sequential signal increases (Δf and $\Delta \theta$) as shown in Fig. 6. A plot of Δf (in Hz), the plateau values, versus target DNA concentrations (Fig. 7) shows

Table 1
QCM-D measurements of the viscoelastic properties ($\Delta D/\Delta f$) of the single-stranded biotin-DNA and formed DNA duplex at different biotin-DNA binding capacity

Biotin-DNA concentration (nM)	Biotin-DNA binding capacity ^a (10^{12} molecules/cm 2)	$\Delta D/\Delta f^b$ (10^{-9} Hz $^{-1}$)		$\Delta m_{QCM}/\Delta m_{SPR}^c$
		Biotin-DNA	DNA duplex	
50	1.2	89	122	6.4
100	2.6	80	113	4.6
200	4.0	77	94	3.9
500	4.5	64	85	4.1
1000	5.9	60	72	3.6

^a Calculated from the parallel SPR measurements using the AutoLab SPR sensitivity of 122 mdegrees = 100 ng/cm 2 .

^b Data at saturated binding.

^c QCM mass is converted from the measured Δf at saturation using the Sauerbrey sensitivity, 1 Hz = 17.7 ng/cm 2 Hz.

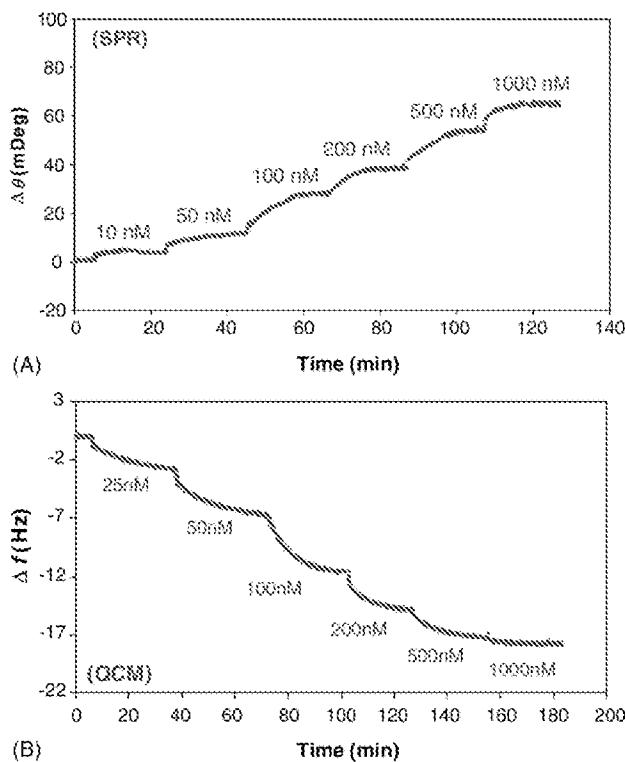


Fig. 6. Titration of surface immobilized biotin-DNA (prepared at 1 μ M) with increasing target DNA concentration: (A) SPR and (B) QCM-D measurement.

a Langmuir adsorption isotherm with a linear response of Δf (Hz) = 0.085C (nM) + 0.317, R^2 = 0.989, up to 150 nM. A similar plot of $\Delta\theta$ (in mdegrees) versus DNA concentrations gives a similar curve with linear response of $\Delta\theta$ (mdegrees) = 0.204C (nM) + 0.700, R^2 = 0.989, also up to 150 nM. From the slopes of the linear curves and the noise of the signal measurements of the instruments (\sim 0.3 Hz for the QCM-D and \sim 1 mdegrees for the AutoLab SPR), the signal-to-noise ratio (S/N) of the QCM and SPR response to a unit DNA loading (e.g. 10 nM) in the DNA hybridization

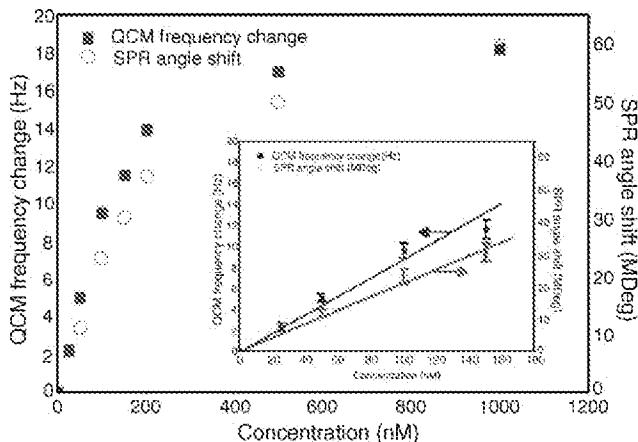


Fig. 7. Plots of SPR angle shift and QCM frequency shift vs. target DNA concentrations. The inset shows the linear response up to 150 nM.

are calculated as 3 (for the QCM) and 2 (for the SPR), which are fairly comparable, although the intrinsic sensitivity factor of the 5 MHz QCM (17.7 ng/cm^2 Hz) is 21-fold lower than that of the SPR instrument (0.82 ng/cm^2 mdegrees). Again considering the noise level, detection limits of both the equipments are about 10–20 nM.

4. Conclusion

SPR spectroscopy and QCM technique, both have their own strengths and weaknesses for the study of macromolecular binding events. SPR is not sensitive to water associated in macromolecules and the signals can be converted to the molecular mass of the adsorbed films in a more direct way. Contrary to that, QCM is not quantitative in this regard, mainly because the QCM measures the molar mass of the adsorbed molecules together with the associated water. With the QCM measurement alone, the contribution of the water to the total mass is not easy to subtract. The complexity in mass measurements, however, does not impair the qualitative evaluation of the probe density effect on hybridization kinetics. In addition, the QCM dissipation provides valuable insight into the film structure, viscoelastic properties, and DNA conformational changes upon hybridization. As for the quantification of target DNA, although the intrinsic sensitivity (signal change induced by unit mass) of the QCM is lower than that of the optical methods, the additional measurements of the associated water in the macromolecules make the actual sensitivity for hybridization analysis comparable with the SPR method.

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Chapter 7

Mass-Sensitive and Thermal Sensors

Learning Objectives

- To be able to explain the principles of the piezo-electric effect.
- To be able to explain how the variation in frequency with mass can be used in sensors.
- To know which materials can be used to produce the piezo-electric effect.
- To know how the piezo-electric effect can be applied in gas analysis and in biosensors.
- To be able to explain the application of piezo-electricity to the quartz crystal microbalance and the electrochemical quartz crystal microbalance.
- To describe the modification of the piezo-electric effect in the formation of surface acoustic waves.
- To understand the variations on the surface acoustic waves principle, i.e. plate wave mode, evanescent mode, Lamb mode and shear mode.
- To know how these variations can be applied to vapour analysis and in biosensors.
- To know the three main types of thermal sensing devices, i.e. calorimetric, catalytic and thermal conductivity.
- To know the construction and operation of a thermistor.
- To describe the application of the thermistor in biosensors.
- To know the principles and applications of the catalytic gas sensor.
- To describe the pellister.
- To describe thermal conductivity devices as used in gas chromatography.

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7.1 The Piezo-Electric Effect

7.1.1 Principles

In 1880, The Curie brothers discovered that anisotropic crystals, i.e. those with no centre of symmetry, such as quartz and tourmaline, give out an electrical signal when mechanically stressed. Conversely, if an electrical signal is applied to such crystals, they will deform mechanically. Thus, with the application of an oscillating electrical potential, the crystal will vibrate.

Every crystal has its own natural resonant frequency of oscillation, which can be modulated by its environment. The usual value of this frequency is in the 10 MHz region, i.e. radiofrequency. The actual frequency is dependent on the mass of the crystal, together with any other material coated on it. The change in resonant frequency (Δf) resulting from the adsorption of an analyte on its surface can be measured with high sensitivity (500–2500 Hz g⁻¹), and when applied in sensors can thus result in devices with pg detection limits.

The relationship between the surface mass change, Δm , and the change in resonant frequency, Δf , is given by the Sauerbrey equation, as follows:

$$\Delta f = -2.3 \times 10^6 f^2 \Delta m / A$$

where Δm is the mass in grams of the adsorbed material on an area A (cm²) of the sensing region, and f is the overall resonant frequency. For a 15 kHz crystal, a resolution of 2500 Hz µg⁻¹ is likely, so that a detection limit of 10⁻¹² g (1 pg) is achievable.

Materials that show the piezo-electric effect and can be used in sensor devices now include ceramic materials such as barium and lead titanates, as well as the 'natural' materials mentioned above. Some organic polymers, such as poly(vinylidene fluoride) (PVDF) (–CF₂–CH₂–CF₂–), also form crystals with piezo-electric properties.

A schematic of a typical arrangement used in a piezo-electric sensor device is shown in Figure 7.1.

SAQ 7.1

- What is meant by the piezo-electric effect?
- Name three materials that display piezo-electric properties.

7.1.2 Gas Sensor Applications

Such sensors are particularly useful for the analysis of vapours and gases that can be adsorbed on to a surface coating on the crystal. Although this method can display a lack of selectivity, specific coatings, however, may selectively adsorb certain gases. Thus, hygroscopic coatings, such as gelatine, silica gel, and

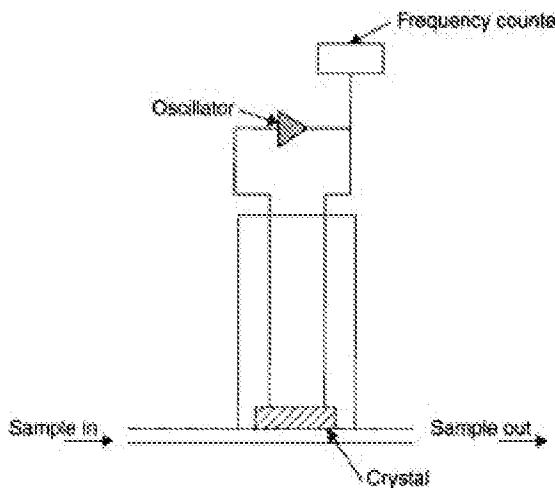


Figure 7.1 Schematic of a typical arrangement used in a piezo-electric sensor device.

molecular sieves, will adsorb water vapour. These substances must be reversibly removable so that the sensor can be used repeatedly. Such sensors have been successfully developed commercially for use as devices for water vapour detection.

Sulfur dioxide is a major pollutant in air, resulting from the combustion of fossil fuels and from motor vehicle engine exhausts. Organic amines will adsorb sulfur dioxide in a reversible manner, according to the following:



However, such amines will also adsorb other acid gases, such as the oxides of nitrogen.

Highly selective sensors have been made for hydrogen sulfide, a very toxic gas with a foul odour, even at very low concentrations. This gas is still toxic at levels that are not detectable by the human nose. Metal acetates, such as those of copper, silver or lead, have been successfully used to detect hydrogen sulfide in sensor devices.

Carbon monoxide is a very dangerous toxic gas, as it has no detectable odour – this gas can often be produced by faulty heating appliances. Although it is not very reactive, its reducing properties have been used to make a piezo-electric sensor. The gas reacts with mercury(II) oxide at 210°C, producing mercury vapour, as follows:



The mercury is then sensed by using a mercury vapour sensor. Mercury is used extensively in laboratories, e.g. in manometers, electrodes and thermometers,

and loss of mercury can therefore result in toxic levels of the material in these environments. Thus, a good sensor for mercury is highly desirable. A device containing a gold-plated quartz crystal has been used to sense mercury, as the latter readily forms an amalgam with gold. Heating to 150°C reverses this reaction.

Various acidic coatings can be used for the detection of ammonia, with ascorbic acid, L-glutamic acid and pyridoxine (vitamin B₆) hydrochloride having all been employed for this purpose. The latter gives a particularly reversible binding with ammonia, plus a high selectivity for this gas. However, other basic analytes, such as amines, can act as interferants.

SAQ 7.2

How is the piezo-electric effect used in sensors?

7.1.3 Biosensor Applications

Much more selectivity is obtained from biosensing methods. Thus, formaldehyde can be detected by coating the piezo-electric crystal with formaldehyde dehydrogenase/NAD⁺ as the selective layer. The enzyme is present in the dry state:



The enzyme and the glutathione cofactor were immobilized on a 9 MHz quartz crystal with glutaraldehyde cross-linking.

Organophosphorus pesticides can be detected by the use of metal salts such as FeCl₃, CuCl₂ and NiCl₂. These will form complexes with such compounds and have been used to make piezo-electric sensors. In addition, cholinesterase enzymes will react selectively with such compounds as 'Malathion'; acetylcholine esterase is immobilized on to a quartz crystal with glutaraldehyde, with a 5 ppm concentration of water being needed to operate this sensor device.

Antibodies make ideal, i.e. highly selective and highly sensitive, coatings on piezo-electric crystals, e.g. 'anti-Parathion' can be used to determine 'Parathion', but again a constant humidity is required. Sometimes, immunosensors are too selective, and thus a more 'general' organophosphorus sensor is needed. Nevertheless, there are many applications in the medical field, where high selectivity is required, such as for immunoglobins, and for *Candida albicans*, a yeast-like fungus which can be found in humans.

7.1.4 The Quartz Crystal Microbalance

It is often more practical to use a differential-mode system, with two balanced crystals and oscillators, as shown in Figure 7.2. Such a system is known as a *quartz crystal microbalance* (QCM). This device is currently receiving

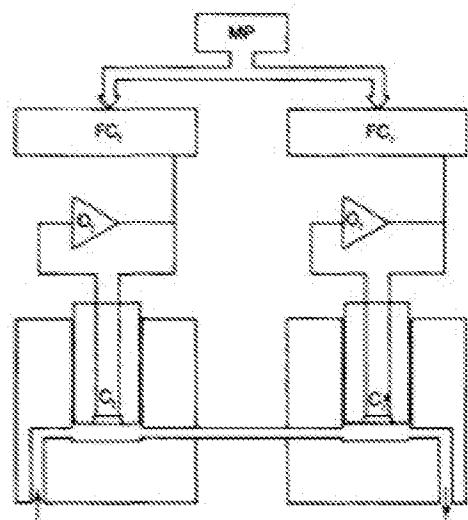


Figure 7.3 Schematic of a typical quartz crystal microbalance system, operating in the differential mode, with two balanced crystals and oscillators, used in electrogravimetric sensor analyses: C, crystal sensor; OC, oscillating circuit; FC, frequency counter; MP, microprocessor; the subscripts 'R' and 'T' refer to reference and test, respectively. Reprinted from *Biosensors: Fundamentals and Applications* edited by A. F. F. Turner, I. Karube and G. S. Wilson (1987), by permission of Oxford University Press.

considerable attention. There have been problems with variability of balance between the two sections and hence a lack of sensitivity and poor signal-to-noise (S/N) ratios. A variation of this uses an amplification scheme – this is referred to as an amplified mass absorbent assay (AMISA). This latter system has been used to measure adenosine 5'-phosphosulfate reductase (APS). In this, an alkaline phosphatase antibody was bound to the microbalance surface, which was then exposed to 5-bromo-4-chloro-3-indolyl phosphate. This caused precipitation of an insoluble dephosphorylated dimer on the balance surface, thus enabling 5 ng cm^{-2} of APS to be detected.

Other applications have been to *Salmonella typhimurium* and to a DNA strand of a *Herpes simplex* virus.

A related device is the electrochemical quartz crystal microbalance (EQCM). A thin layer of a metal such as gold is plated on to the surface of a piezo-electric crystal, which is made the working electrode in an electrochemical cell. The EQCM will detect changes in the mass of material at the electrode, such as (a) adsorption/desorption of species at the monolayer level, (b) electrodeposition/electrodisolution at the electrode due to Faradaic redox processes, and (c) transfer of species between the solution and a surface-immobilizing film.

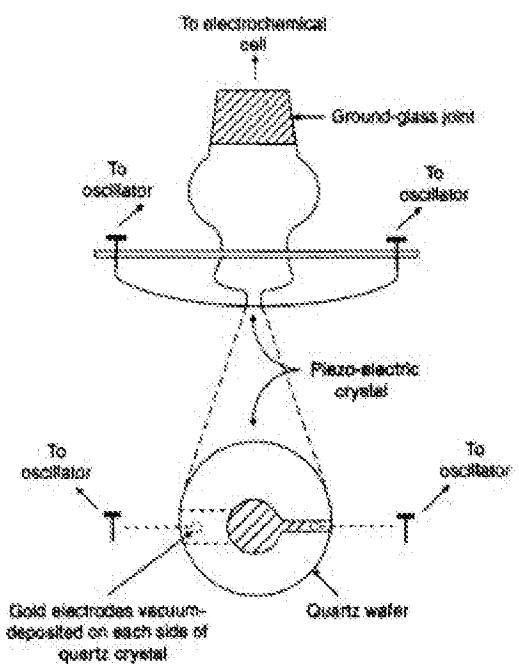


Figure 7.3 Schematic of the electrochemical quartz crystal microbalance. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

A typical EQCM is made from a 10 MHz AT-cut quartz crystal giving a sensitivity of 4 ng Hz^{-1} , as shown in Figure 7.3. It is preferable to compensate for changes in the properties of the solution, such as viscosity, which is affected by temperature, or density. Polymer-modified electrodes can be used, by monitoring the changes in mass measured by the EQCM. Some polymers used in this way include the redox polymers, polythionine and polyvinylferrocene.

SAQ 7.3

How is selectivity obtained in mass-sensitive sensors?

7.2 Surface Acoustic Waves

These are formed by using piezo-electric crystals, particularly lithium niobate (LiNbO_3), although such waves are not generated in the bulk of the solution,

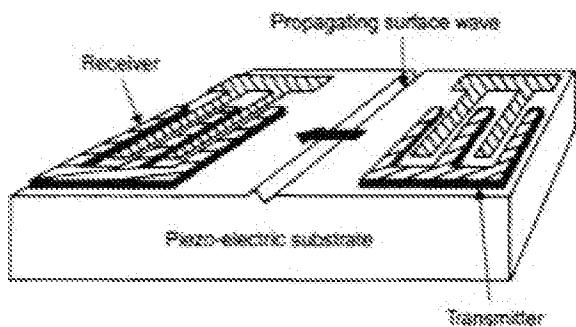


Figure 7.4 Schematic of a surface acoustic wave sensor device. From Zhang, D., Green, G. M., Flaherty, T. and Swallow, A., *Analyst*, 118, 429–432 (1993). Reproduced with permission of The Royal Society of Chemistry.

but on the surface. A transmitter and receiver are positioned at each end of the crystal, as shown in Figure 7.4.

The transmitter and receiver usually consist of sets of interdigitated electrodes. A radiofrequency applied from the transmitter produces a mechanical stress in the crystal, so producing a Raleigh-type surface acoustic wave (SAW) which is received by the second set of electrodes and thus translated into an electrode voltage. The surface wave penetrates into the crystal to a depth of about one wavelength (rather like evanescent optical waves). Thus, a species immobilized on the surface will affect the transmission of the wave, unless the crystal is excessively thick. A number of variations on this technique exist.

7.2.1 Plate Wave Mode

This involves waves being reflected through the bulk of the piezoelectric crystal to an interdigitated transducer (IDT) on one of its surfaces.

7.2.2 Evanescent Wave Mode

This uses a substrate thickness less than the usual five acoustic wavelengths, i.e. down to about two to three acoustic wavelengths, in order to utilize the downward component of the (evanescent) wave. A ground plane is placed between the two IDT structures in order to prevent interference signals.

7.2.3 Lamb Mode

This uses a very small substrate thickness, compared to the wavelength, and the excitation frequency is much lower. Often, an analytically sensitive polymer layer is present on the lower surface, as shown in Figure 7.5.

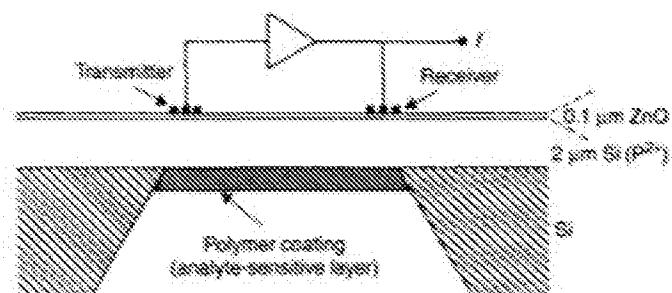


Figure 7.5 A transverse section through a Lamb mode surface acoustic wave sensor device. From Hall, E. A. H., *Biosensors*, Copyright, 1990. © John Wiley & Sons Limited. Reproduced with permission.

This mode offers a higher sensitivity and greater flexibility of sensor design. It has been used with a copper phthalocyanine coating for vapour analysis.

Although a considerable amount of work has been devoted to the study of the properties of these devices, often involving glycerol solutions, very few practical applications have been realized. Those systems studied include the influenza virus and DNA.

7.2.4 Thickness Shear Mode

This mode has been mainly applied to liquids. Extra variables are involved in this case, such as the viscosity, density and conductivity of the liquid being studied. Shear-mode devices generate only two types of analytical signals. First, thin films characterized by a shear modulus of elasticity give rise to standard Sauerbrey mass measurements. Secondly, capture at a liquid-solid shearing surface could lead to a differential signal associated with the introduction of a new material at the interface. Applications have been made of this device towards biosensor development in several ways.

Candida albicans yeasts were detected by means of the anti-*Candida* antibody, which was covalently bonded on to plated platinum electrodes. In addition, human IgG was measured on an AT-cut 9 MHz crystal, modified with protein A being immobilized on an oxidized palladium layer on the crystal surface. Shifts in frequency were ascribed to the affinity of protein A for human IgG.

DQ 7.1

Summarize the relative merits of the different modes of surface acoustic waves (SAWs) for use in sensors devices.

Answer

The basic SAW mode uses Rayleigh surface waves generated by a radio-frequency wave applied to the surface of a piezo-electric crystal, which

then interacts with the surface coating of an analyte species. The wave is detected by a second set of electrodes and converted into a voltage.

In the plate mode, waves are reflected through the bulk crystal to an interdigitated transducer.

The evanescent wave mode involves a lower substrate concentration, and can use the perpendicular component of the acoustic wave.

In the Lamb mode, there is a smaller substrate thickness and a lower excitation frequency. A polymer layer on the lower surface results in greater sensitivity and flexibility of design.

The thickness shear mode is used mainly for liquids, and partially depends on the viscosity, density and conductivity of the liquid being studied. Thin films characterized by the shear modulus of elasticity give Sauerbrey mass measurements. A differential mode variation can also be used.

SAQ 7.4

What detection limits are possible with mass-sensitive sensors?

7.3 Thermal Sensors

7.3.1 Thermistors

A thermistor is a very sensitive device for measuring changes in temperature. Its operation is based on the change in electrical resistance with temperature of certain sintered metal oxides. These include BaO, CaO, or transition-metal oxides such as those of Co, Ni and Mn. There is usually a decrease in resistance of 4–7% per degree rise in temperature, with an accuracy of $\pm 0.005^\circ\text{C}$. Such devices are usually constructed in the form of small glass beads and so can be regarded as miniaturized systems. Thermistors can be used to measure the small amounts of heat evolved in a chemical or biochemical reaction by employing a microcalorimeter. Selectivity can be achieved by carrying out the particular reaction close to the thermistor component, which thus only involves the analyte of interest. More usefully, this technique can be used to measure the enthalpy change in an enzymatic reaction, which, as we have seen earlier, imparts the selectivity. The method can be carried out with coloured or turbid solutions, when colorimetric methods are inapplicable. The enzyme, mixed with albumin, can be coated (immobilized) on to the thermistor by cross-linking with glutaraldehyde, (see Figure 7.6), so making it a discreet sensor. A second thermistor coated only with albumin, acts as a reference. The response is a resistance change, thus giving an electrical signal.

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5

Stable use of biosensors at the sample interface

J. F. GARGHULI, University of London, UK, A. GIEL, and
G. LILLIE, University of Manchester, UK, M. SCHOENLEBER,
University of London, UK, J. PEARSON, University of Manchester, UK,
G. KYRIAKOU and P. VADGAMA, University of London, UK

5.1 Introduction

Biosensors provide a high degree of elegance in regard to their simple juxtaposition of a bioreagent and a transducer function. To work properly, there must be a direct alignment of a functionally responsive bilayer and a transducer element, which is able to directly extract the binding information resulting from the encounter with the analyte. There is a difficulty associated with such a simple, structurally inflexible combination, due to the fact that optimisation is limited as compared with say the use of a liquid phase bioreagents with its attendant optimised solution parameters of pH, pI, concentration and reagent additives.

However, the net result is a solid-state monolithic structure with the potential to perform analyses and to operate potentially in optically opaque samples. In biomedicine, the latter capability holds considerable advantages. Indeed, most if not all biofluids contain colloidal materials that are liable to render the sample optically opaque or, at the very least, to induce a certain amount of light scattering. Furthermore, the majority of clinical sample assays rely on absorbance techniques. Therefore, it is necessary to have access to biosensors that would perform reliably even in opaque samples.

Nonetheless, there are still a few drawbacks associated with the use of biosensors *in vivo*. Although the established biosensor systems operate on the 'macro' scale and have seen varying degrees of clinical exploitation,¹ a key reason for their limited introduction into the application domain has been the rapid alteration of the biosensor interface through the surface activity of the colloidal elements of any unmodified biological sample. This problem is also intricately linked to that of biocompatibility of the exposed surface of the biosensor in direct contact with either living tissues or the physiological fluids present in the body.

5.2 Biosensor limitations

5.2.1 Biosensors and bioresponse compromise

Whilst the bulk amount of protein, colloid and cell transfer to a 'clear' biosensor surface may be relatively low in amount, its diffusional barrier effect on the continued flux of analyte to a responsive biolayer surface will immediately be registered as a reduced biosensor response. The function of any flux dependent biosensor, unless it is merely a qualitative registering device, will be affected sufficiently that accuracy and precision of measurement will be lost, and neither may be recovered simply by recalibration.

The devices therefore likely to be most affected will be those using degrading enzymes rather than where a true binding equilibrium is approached, e.g., antibody, lectins, receptors and DNA/RNA. In the case of the latter group, the only effect should be on the rate of approach to equilibrium not on its final value, unless of course the fouling layer affects solute partitioning. This is possible in principle if, say, the fouling layer is a charged colloid, and the analyte target is also charged; electrostatic forces are then liable to come into play to either partition in or partition out a given analyte. Exclusion, furthermore, is possible if the analyte target is a macromolecule, in which case it may have limited access to the affinity surface of the biosensor through the limited permeability of the colloid fouling layer.

The underlying transducer of the biosensor, whilst not directly affected by surface colloidal deposits, may register the presence of these non-specific elements through its detection domain. Thus, the evanescent wave of an optical wave-guide or SPR system will respond equally to non-specific binding as to specific affinity interactions.

Passivation of the transducer element is possible with some surface-active crystalloids. Thus free amino acids and thiol-containing molecules that are surface active can distort and depress the catalytic behaviour of a Pt working electrode used for redox dependent biosensors.

5.2.2 Biosensors and the selectivity compromise

Whilst the biological component of a biosensor has accepted selective properties, and is *de facto* the driver for biosensor development, the underlying transducer, whether based on electrochemical, potentiometric, optical or microgravimetric principles, is vulnerable to a false positive response due to the surface activity of analogue species of either the target molecule or a molecule that is part of the transduction cascade. The most potent expression of the problem is where an electrically polarised noble metal electrode is used to detect the H_2O_2 product of an oxidase enzyme catalysed reaction in an enzyme electrode. At the typical polarising voltage of +0.65 V vs. Ag/AgCl, numerous species in biological solution are simultaneously oxidised, and false positive responses therefore result.²

5.2.3 Interfacial problems at microfabricated biosensors

The issues of direct biosample interfacing of biosensors applies to all biosensors irrespective of length scale. So whilst the x-y plane architecture may be geometrically precise, formulated in a much more reproductive manner using MEMs and other microforming technologies, the biological response of the host sample reacts in rather similar ways, with adverse buildup along the z axis, i.e., normal to the sensor surface. One rider to this equivalence of macro-microsensor outcomes is where response is flux (continuous diffusion) dependent; a sufficiently small sensing microsurface will have spherical and not a planar diffusion based supply of analyte and is thereby less affected by external variables such as fouling. There is also evidence that a microstructure may set up a lower intensity tissue reaction thus leading to reduced surface fouling.

5.3 Biocompatibility

The term 'biocompatibility' covers the whole range of interactions that exist between a biomaterial implant and its biological surroundings, as well as the orchestrated sequence of responses the body invokes to essentially reject that implant as non-self. In the case of invasive *in vivo* monitoring, an electrochemical biosensor requires intimate, direct contact with the sample matrix in order to function properly, notwithstanding the intensity of the body's reactive response to its constituent materials. As a consequence, the observed performance of the biosensor is highly vulnerable to the local accumulation of surface-active agents from the body such as cells, proteins and other less well identified constituents such as colloidal and lipid aggregates. This accumulation of biological compounds on the surface of the sensing device is known as 'fouling', and will with time alter and degrade the biosensor response and performance. Furthermore, biosensors are not bio-inert, not only because of the active redox components they may incorporate but also because of the polymeric materials, as well as the coated or uncoated metal and carbon electrode interfaces they present to the living tissues. In these tissues, they provoke a high intensity local inflammatory reaction simply because they inhabit a wound site and, in blood, they are a nidus for surface coagulation with the attendant threat of local micro- and later macro-thrombi. The danger of thrombus dissemination in the vasculature, thromboembolism, constitutes a particular concern because of the possibility of considerably wider distribution of tissue damage. There are many examples of implants leading to thrombosis, embolism and death. While the risk might be acceptable for a life-saving device such as a vascular stent, such risk cannot be tolerated when a diagnostic device such as a biosensor is being used.

Even if an implanted material is reputed to have no interaction with a biomatrix and is therefore believed to be bio-inert, some interactions at a microscopic scale still occur. A completely biocompatible material does not

exist. As a consequence, all available materials provoke, to some extent, a biological response, adverse or not, that will impact on overall sensor performance. Indeed, even air entrapped within a tissue will ultimately provoke a local body reaction. Additional bio-effects, beyond local surface phenomena, that need to be taken into account when designing a biosensor for clinical use are carcinogenicity, mechanical stability, immunogenicity, chemical stability and biomechanical compatibility with the local soft tissue. Also, sensors may need to operate *in vivo* for quite different periods of time, and this must be taken into account when assessing the tolerance limit for biocompatibility. Admittedly, in contrast to the carefully assessed quantitative analytical behaviour of sensors *in vitro*, little progress has been made in regard to *in vivo* performance standards and acceptability. Also, the actual limits of tolerance for the degradation of function, resulting from the body's response, are still poorly understood and known.

In vivo biocompatibility can be regarded as a hybrid between biomaterials and biosensor research, which deals with both the specific, as well as the complex interactive and cumulative effects of sample matrix constituents upon sensor function and operational life-time.¹⁻³ For all sensors, understanding and predicting the *in vivo* biological response still poses a great, unmet, challenge. The crucial point is that, in contrast to conventional biomaterials, the surface deposition of the body's diffusive and cellular biocomponents leads to degradation of function, observed within minutes and hours rather than days and months. Whilst the device continues to function, its value as an accurate and precise quantitative system is lost.

A hierarchy of biological interfacial phenomena exist, which, though sub-classified relatively easily, are difficult to unravel in relation to their complex dynamics and as concerted interactive phenomena. For a long time researchers attempted to avoid the bioreaction entirely via the 'mythical' concept of the totally bio-inert implant, leading later to the emergence of the idea of functional biocompatibility.⁴ In this latter case, a primary issue is not so much the total avoidance of the bioreaction, but rather achieving sufficient control over its adverse effects on the biosensor performance.

The definition of biocompatibility is itself an approximation of multiple concepts. However, it is based on the expectation that certain types of materials will be able to provoke just a limited body response. No matter what the material deposited and accumulated at a sensor surface may be, it is whether or not a degradation of response occurs that really matters. The counterpart to this is whether an implant material poses a threat to the patient or not. Both immunological and toxicological dangers exist, for example, through the leaching of additives within covering polymers, the release of polymer degradation products, of metal/metal oxide particulates or of carbon electrode constituents. All are capable of triggering an early inflammatory and toxicological response, but the dangers of long-term effects, including

carcinogenicity are largely uncharted. Again such dangers may be acceptable in the instance of acute life-saving or life quality enhancing implants such as heart valves, aortic grafts and pacemakers, but in the case of a measurement modality such as glucose monitoring, any such dangers have to be negligible.

A biosensor can be regarded as a composite material, usually made up of metallic, polymeric and biological components. While it constitutes a minor burden as an implant, as it exhibits trivial mass and volume in relation to the body, it is however more likely to present a multiple combination of potential leachables, which, together with the reaction products of the biosensing reaction, could have significant local effects. Underlining all this is the fact that any release of antigenic protein poses special dangers as an immunogenic trigger, especially if a biosensor is to be implanted repeatedly. The biocomponent (e.g. enzyme) may itself be associated with an undefined constituent due to the presence of low level impurities, as in any biogent, rendering traceability difficult. A practical expression of this comes from the routine use of bovine serum albumin (BSA) as a crosslinking matrix for glucose oxidase in the early days of research.⁷ Due to actual and theoretical dangers of the bovine spongiform encephalitis (BSE) agent, such a protein source for enzyme immobilisation is no longer acceptable. In view of the heterogeneity of both sensor internal structure and of the multiple types of surfaces presented to the biomatrix, the body's response maybe quite different over the active sensing regions of the device vs. the support regions. This makes it difficult to determine a precise structure - biocompatibility linkage.⁸

Materials considerations are also relevant to the duration of operation envisaged. Short-term monitoring in, say, the critically ill patient, demands quite a different level of materials requirement to the more robust, mechanically resistant devices that, moreover, would have to be well tolerated by the patient over the long term, especially during ambulatory monitoring. Polymer encapsulant degradation and metal corrosion set a limit to the long-term implantation of the sensor in the patient. Furthermore, not only are the body's responses cumulative but a rigid device, mechanically incompatible with local soft tissue, will not be well tolerated, due to the stimulation of local pain sensors. Over time these released constituents, due to the immune response, will be upregulated; so will be the effects of unavoidable products of enzyme reactions and redox centre/modulator components.

Wherever a foreign body is lodged in the tissue or the blood compartment, it can also serve as a focus for infection and poses a problem over the long term. Microbial films may form on the surface and these have a powerful way of resisting antibiotic assault. Such films are known as significant contributors to hospital infection rates.⁹ The situation is compounded if a device is only partially inserted as with, say, a percutaneous glucose needle sensor. These are typically implanted in vascular, partly fatty tissue to only a 10–15 mm depth. Whilst less invasive, they provide a contact pathway for externally derived

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microorganisms. Important in this regard is the fact that the skin surface has its own microbiological flora and cannot be fully sterilised.

Diffusible, low molecular weight solutes in biological fluids provide a nominally stable solution environment *in vivo*, given that this internal environment is designed for physiological stability. Certainly, near-neutral pH conditions normally prevail, and the concentration variation of background electrolytes is relatively small, at least with regard to the major ions such as sodium, potassium and calcium. As an indicator of the total solutes, osmolality in blood ranges quite narrowly between 280–295 mOsm/kg. With the inherent stability of the enzyme within an electrochemical biosensor and its reduced dependence upon pH and background ionic changes due to the immobilisation, one would expect minimal background solute effects upon bioelectrochemical sensor function. However, even small variations will cause some analytical imprecision, especially as sample dilution or other specimen manipulation are precluded in contrast to bench top analysis.

The above considerations have been a basis for the use of, say, sampling tissue ultra-filtrate for glucose monitoring via mechanical suction through permeabilised skin.¹⁰ However, low molecular weight solutes may still influence working electrode response through adsorption, passivation and then direct surface activity. Such effects may be difficult to identify when conducting measurements in a complex medium such as blood or tissue combining macro- and microsolute influences. The former are only external surface active; the latter are able to permeate the entire device. The adsorbed solute can induce degradation of metallic components, as described for electrodes used in electrical stimulation, where increased ionic release and accelerated corrosion have been reported¹¹ with a further facilitating influence due to surface active proteins.¹² The electrochemical reaction can also contribute to electrode dissolution, which is of relevance to long-term implantation.¹³

Beyond standard cylindrical geometries and wire-type devices, emphasis is shifting to MEMs-based devices. However, some implantable sensors based on MEMs constructs are vulnerable to hydration and water ingress. As a consequence, rigorous packaging and encapsulation are needed for long-term function as well as over the short term. Typically, in order to avoid leakage currents and extraneous responses, inorganic or polymeric packaging materials are required.^{14,15}

5.3.1 Protein constituents

In terms of the total colloid biomass, proteins comprise the most important surface-active constituents of biofluids other than viable (and dead) cells. Total concentration in plasma is around 80 g/l. Proteins have a special ability to adsorb physically at solid/liquid interfaces and through consequent denaturation to form relatively adherent, immobilised phases, showing only partial remodelling or

recycling between the bulk sample and surface. The adsorbed proteins thus undergo both conformational and orientational changes at the surface. As well as progressively increasing in depth with time, through deposition of solution originated proteins, the entire protein 'gel' layer grows unpredictably, modulated by both environmental perturbation (convection, shear, pressure) and inherent structural remodelling, especially evident near the growth surface.¹⁶ Again, the key issue is that in contrast to conventional biomaterials, surface deposition effects on a new electrode surface are rapid and accumulated. Whilst the device continues to function, its value as an accurate and precise quantitative system is almost immediately lost, a particular concern in the case of glucose monitoring given the strict control demanded in diabetes management.

Protein deposition occurs within seconds of contact,¹⁷ but inherent film composition and individual component abundance are conditioned by material surface energy (hydrophobicity/hydrophilicity), charge, charge density, surface profile and roughness, degree of molecular ordering, pendent group flexibility and crystallinity. In the case of a polymeric interface, the presence of trace components, plasticiser content and minor degrees of surface oxidation all drive particular types of protein adsorption.

Albumin, in particular, is a dominant constituent at the interface, and outcompetes other proteins in plasma such as immunoglobulins, fibrinogen and kininogen. Its presence at a surface, furthermore, helps to reduce the deposition of other surface fouling components including cells, and it is regarded as a passivating protein. The dynamics of the adsorption, however, appears to be different at different surfaces. For example, on a hydrophobic surface, adsorption is a single-step process, whereas on a hydrophilic surface, it involves two steps. The resultant in either case, though, may be the formulation of an equivalent layer with similar passivating behaviour.¹⁸ This new view of protein deposition reduces the importance of the primary surface in driving biocompatibility outcomes. Ongoing competition between proteins for the surface leads to the remodelling of the deposited surface layer and, in the context of, say, fibrinogen adsorption from plasma, its transient surface prevalence and subsequent partial displacement (the Vroman effect) represents the outcome of the finite number of available sites on the surface.¹⁹

In previous studies, surfaces have been alkyl modified in order to promote albumin deposition, and therefore to reduce platelet adhesion.²⁰ More recently, immobilisation of highly hydrophilic polymers such as poly(ethylene oxide) (PEO) has proved especially valuable in resisting protein deposition.²¹ The equivalent has also been achieved, for example, through glow discharge deposition using tetramethylene glycol dimethyl ether.²² More classically, heparin immobilised at surfaces has proved effective in resisting a special type of surface deposition, the binding and activation of coagulation components. In this respect, surface bound heparin has been used to good effect at an intravascular oxygen catheter sensor.²³

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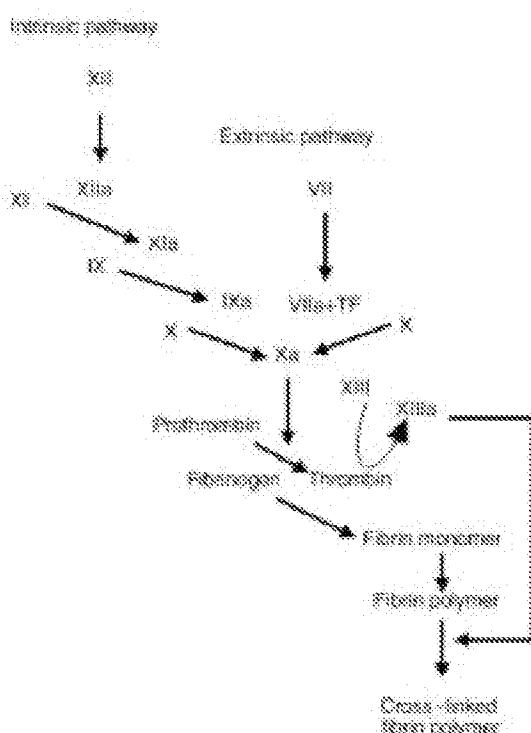
Heparin works through its anionic sulphonate and aminosulphate groups,²⁴ so attempts have been made to sulphinate artificial polymers, e.g., polystyrene and polyurethane.²⁵ Also, the combined effect of PEO with end-attached sulphates to give heparin-like properties, and the added benefits of PEO flexibility has led to an improved thrombo-resistant polyurethane.²⁶ Polyurethane is already frequently used as an outer membrane barrier for glucose sensors, so this may have a direct application when used in a bioelectrochemical sensor.

The fundamental drawback of an enzyme-based biosensor is that it requires continuous substrate flux to the enzyme layer for an ongoing response. This flux has to be purely substrate concentration (gradient) dependent, unperturbed by any newly formed diffusion barrier in, on or around the biosensor itself. The first of the membrane-packaged devices developed to control such adventitious biolayer diffusion limitation was the Clark pO_2 polarographic electrode where an external gas permeable membrane served to protect the working electrode from protein and colloid deposition.²⁷

For surface anticoagulation, a primary need is for the prevention of the binding of factor XIII, as this is a key trigger in the initiation of the coagulation cascade and eventual deposition of the crosslinked fibrin mat at the surface. An intrinsic pathway and an extrinsic coagulation pathway, the latter being induced by tissue-derived factors, may combine to create an accelerated cascade of fibrin deposition (Fig. 5.1). However, a parallel system of surface-active proteins has been rather neglected. This is the complement system, which undergoes an ordered, sequential response to an artificial surface, eventually to trigger the production and the release of inflammatory mediators from white cells.²⁸ This complement activation takes place through one of two pathways, the classical and the alternative.

The classical complement pathway is initiated by antigen-antibody complexes, by crystals or bacterial and virus surfaces if antibodies are absent, or by complexes between positively and negatively charged molecules such as those between heparin and prothrombin. The alternative pathway is not triggered by immune/antibody complexes, but can be initiated by any foreign material introduced into the body, including a biomaterial, lipopolysaccharide, polysaccharide, or bioorganism. It is activated by surfaces with particular chemical characteristics allowing fragment C3b of a larger protein C3 at the surface to initiate the assembly of an amplification system, C3 convertase, at the surface²⁹ for further C3b deposition (Fig. 5.2).

With regard to specific sequences, C3 convertase cleaves C3 to generate C3a, and a further fragment C3b. In the nascent state, the latter binds to the surface and augments the convertase enzyme further to amplify C3b deposition on the surface. A further reaction leads to the cleavage of available C5, with the production of a C5a fragment and also recruitment of a C5-C9 sequence of effectors and associated inflammatory changes. It is clear that substantial complement activation can lead to major organ dysfunction, though admittedly

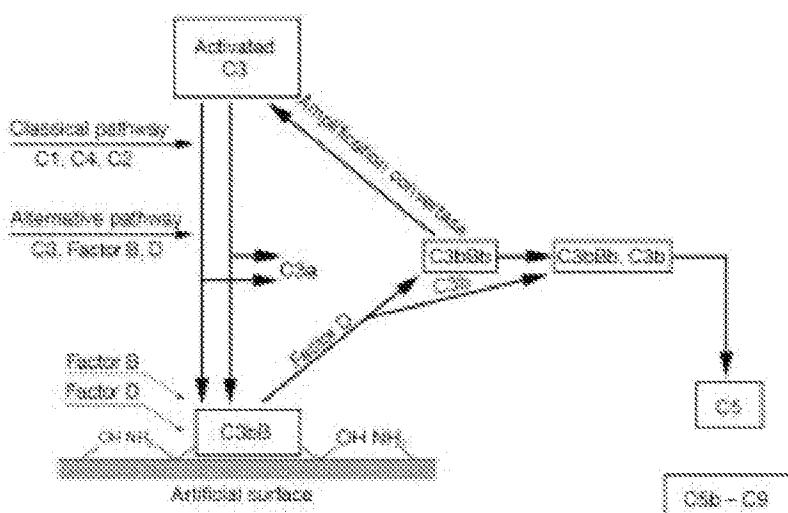


5.1 Surface coagulation cascade initiated either by surface contact (the intrinsic pathway) or by tissue factor (TF, the extrinsic pathway). The two pathways are eventually converging, forming a fibrin clot due to activation of thrombin on fibrinogen. Factor XIII will eventually convert fibrin clot into insoluble fibrin gel.

only following large scale blood/surface interactions as in haemodialysers.²⁹ However, this complex cascade also requires to be considered as a possible contributor to local events at the biosensor surface. Surface amines and hydroxyls, in particular, react with C3 to form complexes, though there is uncertainty whether these are covalent bonds or whether electrostatic and hydrophobic interactions are important.³⁰ Outcomes with regard to systemic effects also need to be unravelled.³¹ Local effects of relevance to a biorelectrochemical sensor might be abated through control of the surface presented, perhaps through surface heparinisation.³²

5.3.2 Blood interfacing

Blood consists of about 55% plasma by volume with about 45% solid particles including proteins and cellular elements, especially red blood cells, but with a



6.2 Surface induced complement activation leading to accelerated complement protein deposition at a surface expressing amino/hydroxyl groups. Sds cascade generation of C3-C9 is also indicated.

small white blood cell and platelet volume contribution. Red blood cells contain mainly haemoglobin, carry oxygen to the peripheral tissues and normally do not leave the circulation. White blood cells, however, are able to leave the vascular system to move towards any active disease tissue focus from, say, that due to microorganisms intrusion through a foreign body. The induced tissue disruption is "sensed" very acutely, no matter how localised. Additionally, platelets operate as a natural blood containment system, typically by forming a mechanical micropore at a vascular injury site, eventually producing a defined clot to contain blood in the circulation.

The cellular elements of blood play a complex, cooperative role in the maintenance of the surface coagulation process initiated by soluble coagulation factors (*vide supra*). However, the deposited protein layer from blood at the sensing surface changes the material interface considerably, and it is this layer over which the cellular elements then begin to accumulate. Initially, the most important of these are platelets, which normally circulate in the blood in an inactive state, but are also the most labile of all the formed elements and therefore the most difficult to evaluate in physiological studies. Once they come into contact with a foreign surface, they show strong adhesion. This spreading and aggregation behaviour, associated with the release of intra-platelet adenosine diphosphate (ADP), promotes secondary platelet aggregation and the creation of (adhesive) thrombus. In the final analysis, the level of initial coagulation protein deposits at a surface are seen to correlate with platelet

surface activity and therefore, both condition the blood compatibility of a given material.³³

Quite apart from any surface effects, platelets are exquisitely sensitive to shear force. Both the magnitude of shear and the overall blood flow profile near a surface, through influence on platelet transport, can completely override surface affinity interactions. Moreover, turbulent conditions and their associated high shear are particularly able to trigger platelet activity and augment surface delivery.³⁴ The platelet delivery process is further accentuated by red cell interactions and local fluid entrainment around red cells, promoting surface collisions.³⁵ The net negative charge on a platelet, due to surface sialic acid groups, is important to surface attachment. However, surface receptor interactions are also a powerful determinant, such as the receptor mediated attachment of platelets to fibrinogen. Following adhesion, platelets degranulate with the release of ADP. Then, a host of other biocactive components are able to promote further platelet activation and the eventual creation of an adherent micro-thrombus.

Incorporation into the thrombus of the other cellular components of blood, including white cells and red cells (both influenced by blood flow and pressure gradients), leads to thrombus growth. As a consequence, local blood flow is distorted near the originally smooth surface, further stimulating the growth of thrombus and aggregation of blood components of all types. The effect on a sensing surface is the reduction of solute transport to that surface and the local consumption of oxygen/glucose, depending upon the metabolic state of the cellular aggregates, thereby inducing changes on the locally measured parameters.

It becomes especially difficult to characterise material-induced artefactual influences upon a sensor, let alone to model them. Therefore, their subtraction from true responses *in vivo* becomes problematic, demanding at the very least a frequent *in-vivo* calibration regimen. It may be that, in the future, the quantity and nature of a surface coagulation phase can be measured using an independent technique. Impedance measurements promise in this regard to allow at least for baseline drift³⁶ but, in reality, the end result at present is the highly unsatisfactory need to recalibrate frequently.

The imposed structure of an intravascular electrode disrupts blood laminar flow patterns and can be an indirect cause of surface thrombus formation. Experience with thrombotic events and clinical use of intravascular catheters indicates a relationship between vessel cross-section diameter and catheter size.³⁷ Complicating the assessment of protein deposition, coagulation/complement activation and platelet resealing,^{38,39} surface irregularity and surface microdepressions over a device may promote thrombus formation.^{39,41} A smooth surface is an advantage, but, probably, surface anticoagulation is a more effective basis for reducing thrombus formation. There is a clear lesson here for the combination design of smooth, haemodynamically acceptable glucose sensor surfaces and their surface modification.

Heparin is a natural anticoagulant in blood, and is the most frequently used bioactive surface ingredient used to reduce clotting. The protective action of heparin is based upon its stimulatory effect on antithrombin via an heparin-antithrombin complex, though this may be countered by the fibrin interactions of thrombin.⁴² Surface heparinisation of membranes has allowed more reliable oxygen monitoring.⁴³ Catheter heparinisation has also proved to be of general effectiveness.⁴⁴ Covalent binding of heparin, though permanent, leads to a more rigid attachment which can reduce effectiveness so bridging groups are an advantage. Also, depending upon the required duration of sensor operation, heparin leaching from a porous membrane or other reservoir could lead to a more potent anticoagulation surface. Overall, heparin can play a major role in reducing the effects of coagulation, but it is not the complete solution to the problem hoped by some. Alternatives to heparin include low molecular weight anionic analogues and, in particular, poly(ethylene oxide) (PEO).⁴⁵ Furthermore, the use of copolymer structures rather than surface attachment may provide a family of new blood-stable materials in the future.⁴⁶

5.3.3 Tissue interfacing

Subcutaneous tissue is a safer alternative to the intravascular siting of glucose sensors, avoiding the dangers of thromboembolism, as well as of rapid dissemination of infection. Problems of reliable monitoring, though different from those of blood, are nevertheless substantial. The implanted device, through its intrusion into a normal tissue architecture, is perceived by the body (as what it is, a disruptive foreign body). As a response, the tissue sets up an intense (acute) inflammatory response designed to degrade, isolate and ultimately reject the foreign material.⁴⁷

The outcome is locally distorted body fluid composition, i.e., modified functional physiology. As a consequence, no matter how reliable or biocompatible the sensor may be, measurements are thereby performed in an environment that is metabolically distorted and also appears to lose the rapid equilibrium relationship with the local blood and the capillary bed supply (Fig. 5.3). With a low-reactivity material, the acute inflammatory stage subsides to give way to tissue repair, which tends to generate a more vascular environment through capillary proliferation, as well as a matrix rich in fibroblasts. Capillary density in the vicinity of implanted electrodes has been shown to increase with a vascular density maximum at a 50 µm distance from the sensor.⁴⁸ However, measurement distortion was observed (sensor instability and slow response) and was thought to be due to local fluid increase. Whatever the mechanism, the distorted response was only marginally diminished for a tissue-implanted glucose sensor by using slow release of dexamethasone as an anti-inflammatory.⁴⁹

Due to the presence of soluble bioactive agents, the tissue environment becomes highly hostile to the sensor. Local increases in hydrolase enzyme

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LEXSEE 189 USPQ 143

IN THE MATTER OF THE APPLICATION OF VERNE R. RINEHART

Patent Appeal No. 75-608

UNITED STATES COURT OF CUSTOMS AND PATENT APPEALS

531 F.2d 1048; 1976 CCPA LEXIS 185; 189 U.S.P.Q. (BNA) 143

March 11, 1976, DECIDED.

PRIOR HISTORY: [**1] Serial No. 130,743.

LexisNexis(R) Headnotes

OPINION BY:

MARKEY

OPINION: [*1049]

MARKEY, Chief Judge.

This is an appeal from the decision of the Patent and Trademark Office Board of Appeals (board) affirming the examiner's final rejection of claims 1 through 9, which are all the claims in appellant's (Rinehart's) application serial No. 130,734, filed April 2, 1971 n1 entitled "Process for Preparing Resin." We reverse.

n1 The present application is a continuation-in-part of application serial No. 667,854 (parent), filed September 14, 1967, which in turn is a continuation-in-part of application serial No. 254,754, filed January 29, 1963, both of which are now abandoned. Prior to the present appeal, the rejection of parent application was appealed to the U.S. District Court for the District of Columbia. Goodyear Tire & Rubber Co. v. Schuyler, Com'r., Civil No. 666-71 (D.D.C., Feb. 25, 1975). Upon stipulation, that action was dismissed with prejudice, after the express abandonment of the parent application, but without prejudice to the allowance of materially

different claims, or of the same or similar claims on a record supporting them, such as the record now before us.

The Invention

Commercial [**2] scale quantities of polymeric ethylene terephthalate (PET) are produced in either a batch or continuous process by heating a dicarboxylic acid with glycol in the presence of a preformed low molecular weight polyester solvent n2 under superatmospheric pressure and utilizing a low ratio of glycol to acid. The product may be conventionally condensation polymerized in the presence of a catalyst.

n2 The solvent may include stabilizer, catalyst, and ether inhibitors.

The claims have been treated together by Rinehart and the solicitor and will be so treated here. Claims 1 and 4 are illustrative:

1. The method for the commercial scale production of polyesters which comprises adding commercial scale quantities of ethylene glycol and a free aromatic dicarboxylic acid in the molar ratio of glycol to acid of from 1.7:1 to 1.05:1 to a solvent consisting of a preformed low molecular weight linear condensation polyester of a glycol and a dicarboxylic acid, said polyester having an average degree of polymerization of from 1.4 to 10, heating and reacting the mixture at a temperature above the melting temperature of the low molecular weight linear polyester at a pressure of from about 20 [**3] to about 1000 pounds per square inch gauge pressure until a linear condensation polyester resin

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of said glycol and acid having an average degree of polymerization of from 1.4 to 10 is formed.

4. The method for the commercial scale production of polyesters which comprises continuously adding commercial scale quantities of ethylene glycol and terephthalic acid in the ratio of from 1.7:1 to 1.05:1 of ethylene glycol to terephthalic acid to a solvent consisting of low molecular weight ethylene glycol-terephthalate polyester having an average degree of polymerization of from 1.4 to 10 while heating and reacting the mixture at a temperature above the melting temperature of the low molecular weight ethylene glycol-terephthalate polyester at a pressure range of from about 20 to about 1000 pounds per square inch gauge pressure, continuously venting the water vapor formed in the reaction at such a rate that the pressure in the system is maintained constant within said pressure range and continuously withdrawing an amount of low molecular weight ethylene glycolterephthalate polyester about equal to the amount of ethylene glycol and terephthalic acid added.

Board

The board affirmed the **[**4]** rejection of claims 1 through 9 under *35 USC 103* as **[*1050]** obvious on Pengilly n3 and Munro et al. (Munro) n4 "considered together." n5 Both Pengilly and Munro form PET by heating, in either a batch or continuous process, a dicarboxylic acid with glycol, utilizing low ratios of glycol to acid (for example, 1.05:1.0 to 1.3:1.0 for Pengilly), and then polymerizing the low molecular weight ester formed therefrom in the presence of a catalyst. The processes differ in that the initial step of the Pengilly process is conducted at atmospheric pressure utilizing a preformed polyester solvent, whereas Munro operates at a higher pressure absent the solvent.

n3 U.S. Patent No. 3,427,287 issued February 11, 1969.

n4 U.S. Patent No. 3,050,533 issued August 21, 1962.

n5 The board also affirmed a double patenting rejection of those claims under *35 USC 101* based upon the copending parent application.

Express abandonment of the parent application, subsequent to the board's decision, moots the issue.

The appealed claims differ substantively from those of the parent application only in reciting "commercial scale production" utilizing "commercial scale quantities." Because the **[**5]** claims in the parent application had been rejected under *35 USC 103* on the same prior art and logic, the board merely adopted the previous board opinion, which held that the references established a case of "prima facie obviousness." The earlier board, agreeing with the examiner that Pengilly and Munro considered together rendered the claimed subject matter *prima facie* obvious because each suggested consonant advantages, stated:

For example, Pengilly suggests that by using a polyester solvent shorter heating times and less glycol is required, and Munro et al suggests that by using higher pressures a shorter reaction time is required. One of ordinary skill in the polymer art would therefore except that if higher pressures were used in other art processes (i.e., Pengilly) shorter reaction times would be necessary.

n6

n6 The earlier board also speculated that Munro's continuous process may "actually involve the use of preformed ester as the reaction solvent if the reaction takes place throughout the reactor and if, during the initial part of the process, the product is not withdrawn as rapidly as it is formed."

The board considered the rebuttal evidence, a single affidavit **[**6]** by the inventor, Rinehart, to be insufficient. The primary apparent purpose of that evidence was to show the commercial inoperability of Pengilly and Munro, taken individually, compared to Rinehart's commercially used method. Rinehart's extensive affidavit included, however, substantial analysis of the entire field of polyester production and of what, in his view, Pengilly and Munro would actually teach those skilled in the art. The experimental pilot plant evidence is summarized below for a low charge molar ratio of glycol to acid (1.1:1.0):

	Esterification Reaction			
	1 Munro	2 Rinehart	3 Pengilly	4 Munro
Pressure (psig)	40	40	0 (Atmos.)	40
Temperature (degree C.)	250-261	248-252	*	260-262
Reactant Batch				
Size (pounds)	122.1	122.1	122.1	268.6
[Solvent]/Batch	No [Solvent]	1.2/1.0	1.2/1.0	No. [Solvent]

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	Esterification Reaction			
	1 Munro	2 Rinehart	3 Pengilly	4 Munro
Average Time (Min.)	330	150	657	483
% Ether	2.99	1.68	1.51	3.08
Melting Point	244.9	252.2	252.8	244.1
Gardner Rd	27.1	24.9	27.0	25.4
Gardner b+	14.0	8.3	13.6	17.8

* The temperature was increased at a rate of 3 degrees C/30 minutes from about 220 degrees C to 245 [**7] degrees C. [*1051]

Rinehart alleged commercial success, based on the 1970 conversion by Goodyear Tire and Rubber Company (the assignee of Rinehart) from the ester interchange method, used since 1959, to Rinehart's direct esterification method.

The affidavit states:

Both the Pengilly, and Munro and Maclean, procedures based on my experience and as evidenced from their patents are operable on a small scale. However, neither of their patents points to any recognition of the problems which arise from scaling up to a commercial process. It is implicit in their patents that the described procedures are satisfactory for commercial operation; but I have found that their techniques are not satisfactory on a commercial scale at about equimolar proportions. The advantages claimed by Munro and Maclean for their process are a short reaction time, improved color, higher softening point and a minimum ether content. However, I have found that as the Munro and Maclean process is scaled up beyond laboratory equipment the reaction becomes inconveniently long, the color deteriorates, the melting point is lowered and the ether content increases. The process of Pengilly was similarly operable [**8] on a small scale and not suitable for scale-up to a commercial process.

The board concluded that the affidavit evidence did not rebut its finding of prima facie obviousness because, in its view, the prior art clearly suggested higher pressure, together with an expected attendant advantage of increased reaction rate, as a solution to the commercial difficulties allegedly encountered by Rinehart. Moreover, the recitation to which the affidavit is directed, "commercial scale production" utilizing "commercial scale quantities," was viewed as "inherently" obvious. The board did not consider the utilization of the claimed method by Rinehart's assignee to be evidence of commercial success sufficient to establish unobviousness.

Issue

Whether, in the light of all the evidence, the claimed

method would have been obvious at the time the invention was made.

OPINION

Pengilly and Munro individually teach methods for the production of PET which differ, in different respects, from that claimed by Rinehart. A determination under 35 USC 103, however, requires consideration of the entirety of the disclosure made by the two references to those skilled in the art.

A prima facie case [**9] of obviousness is established when the teachings from the prior art itself would appear to have suggested the claimed subject matter to a person of ordinary skill in the art. Once such a case is established, it is incumbent upon appellant to go forward with objective evidence of unobviousness. *In re Fielder*, 471 F.2d 640, 176 USPQ 300 (CCPA 1973).

Prima Facie Obviousness

On the appeal involving Rinehart's parent application, the board was limited to the sterile evaluation of the claims and the prior art necessitated by availability of only the application and the cited references. Based on that evaluation, that board stated:

We agree with the examiner that, in view of Munro et al., it would be obvious to operate the process of Pengilly at superatmospheric pressure. Looking at it from another point of view, it would be obvious in view of Pengilly to employ preformed ester as a solvent in the reaction of Munro et al. [*1052]

On the appeal of the present application, the board stated:

With regard to the rejection under Section 103, we find ourselves in substantial agreement with the position of the examiner as set forth in his answer. The claims on appeal are in essence [**10] the same as those in Serial No. 667,854, which is now before the District Court for the District of Columbia (Civil Action 666-71), the basic difference being the involved claims recite and are limited to "commercial scale production" utilizing "commercial scale quantities." The claimed invention is otherwise identical insofar as the material limitations defined are concerned. The claims in parent case Serial No. 667,854 were rejected under Section 103 over the

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same art applied herein and essentially for the same reasons. Insofar as the question of whether or not the combination of the teachings of Pengilly and Munro et al would render the claimed process *prima facie* obvious, the same arguments were presented by appellant and the examiner in both the prior case and herein. Based on these arguments, the Board of Appeals agreed with the position of the examiner and affirmed the rejection. Appellant has set forth no good and sufficient reason why we should reconsider the prior Board decision or reach any other conclusion based on the arguments alone; we therefore adhere to that position and adopt it as our own.

The only remaining question for this Board to consider with regard [**11] to the Section 103 rejection is whether or not the affidavit filed under the provisions of Rule 132 is sufficient to rebut the *prima facie* case: in our opinion, it is not.

The board erred in adopting the earlier opinion. The basis for evaluation and for decision had changed. The present board had before it not only the application and the prior art but all of the unrebutted facts established in Rinehart's affidavit. At that stage no question of *prima facie* obviousness remains. The appealed claims must be reconsidered in the light of all the evidence, and the resultant finding, that the claimed invention would or would not have been obvious, is to be made in such light.

The concept of rebuttable *prima facie* obviousness is well established. Cf. *In re Freeman*, 474 F.2d 1318, 177 USPQ 139 (CCPA 1973); *In re Klosak*, 59 CCPA 862, 455 F.2d 1077, 173 USPQ 14 (1972); *In re D'Ancicco*, 58 CCPA 1057, 439 F.2d 1244, 169 USPQ 303 (1971). It is not, however, a segmented concept. When *prima facie* obviousness is established and evidence is submitted in rebuttal, the decision-maker must start over. Though the burden of going forward to rebut the *prima facie* case remains with the applicant, [**12] the question of whether that burden has been successfully carried requires that the entire path to decision be retraced. An earlier decision should not, as it was here, be considered as set in concrete, and applicant's rebuttal evidence then be evaluated only on its knockdown ability. Analytical fixation on an earlier decision can tend to provide that decision with an undeservedly broadened umbrella effect. *Prima facie* obviousness is a legal conclusion, not a fact. Facts established by rebuttal evidence must be evaluated along with the facts on which the earlier conclusion was reached, not against the conclusion itself. Though the tribunal must begin anew, a final finding of obviousness may of course be reached, but such finding will rest upon evaluation of all facts in evidence, uninfluenced by any earlier conclusion reached by an earlier board upon a different record.

The board's analytical process appears to have resulted, at least in part, from Rinehart's erroneous

argument that the mere inclusion of "commercial scale production" and "commercial" scale quantities" served to patentably distinguish the appealed claims over those in the parent application. In response, the [**13] board engaged in comparison of the two sets of claims and emphasized their essential identity. Whether engendered by Rinehart's arguments, the concentration on the "inherent obviousness" of scaling up led Rinehart and the solicitor into error.

Rinehart erred in contending that the mere insertion into the claims of "commercial" [**1053] scale," without more, would constitute a distinguishing limitation. Though inclusion of the phrase in the claims does no harm, it is clear that mere scaling up of a prior art process capable of being scaled up, if such were the case, would not establish patentability in a claim to an old process so scaled. Moreover, absent evidence to the contrary, nothing in Pengilly or Munro indicates that their processes are not effective on a commercial scale, and Rinehart concedes that commercial operation is implicit in the reference patents.

Rinehart argues here that merely because the appealed claims include a "crucial limitation" to commercial quantities, they were "different claims" and that the board could not therefore have applied the earlier decision to them. We cannot agree. Absent the evidence in Rinehart's affidavit, use of commercial quantities [**14] in the processes of the references would have been obvious. If all Rinehart had done was to add the broad "commercial scale" phrases, the board's treatment would have been correct. It could not have found that the mere use of commercial quantities established unobviousness of the invention as a whole. But Rinehart did more. He submitted substantial evidence touching the basic question of whether his claimed process would have been obvious.

The board erred, as above indicated, in comparing the appealed claims to the earlier claims as though it had been established that the latter did in fact set forth an old or obvious process. In such comparison, the board proceeded as though the earlier claims were a kind of prior art to Rinehart and as though the earlier decision on those claims was a kind of *res judicata*. The differences between the two sets of claims were simply not at issue in this case. The sole question is whether Rinehart's claimed process would have been obvious in view of all the evidence.

The Evidence

The opinion of the board on the appeal involving the parent application included the following:

Appellant alleges the existence of numerous difficulties with [**15] the processes of Pengilly and Munro et al. which, he claims, are overcome by combining the features of both processes. However,

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appellant's allegations are not supported by any evidence.

The evidence now or record, in our view, does support Rinehart's allegations. The assertion that the processes of Pengilly and Munro cannot satisfactorily be scaled up is neither challenged nor rebutted. Though mere reference to "commercial scale quantities" in the claims and affidavit does not itself establish patentability, it does establish the environment of the invention. It outlines the problem solved and gives dimension to Rinehart's contribution. The claims must therefore be considered, and the references must be evaluated, in the light of an effort to achieve commercially effective production. As will appear hereinbelow, the affidavit evidence also spotlights portions of the prior art disclosures indicating unobviousness of the claimed process.

It is true that Pengilly and Munro both disclose processes for polyester production by direct esterification. Rinehart's affidavit admits that he began with an effort to employ the process of Pengilly on a commercial scale and that the [**16] only essential difference between the claimed process and that of Pengilly is the employment of superatmospheric pressure.

The board adopted the earlier opinion, which considered the claimed process as either that of Pengilly with the substitution of the superatmospheric pressure disclosed by Munro or that of Munro with the use of a preformed polyester as disclosed by Pengilly. But that view of the claimed process does not end the inquiry. The question remains whether it would have been obvious, in scaling up Pengilly's process, to have employed Munro's higher pressures or in scaling up that of Munro to have employed Pengilly's preformed polyester.

The tribunals below did not meet the requirement of establishing some predictability [**1054] or success in any attempt to combine elements of the reference processes in a commercial scale operation. As in *In re Naylor*, 54 CCPA 602, 369 F.2d 765, 152 USPQ 106 (1966), we find nothing in the record which would lead one of ordinary skill to anticipate successful production on a commercial scale from a combination of such elements, without increase in glycol-acid ratio. The record in fact reflects the contrary. The view that success [**17] would have been "inherent" cannot, in this case, substitute for a showing of reasonable expectation of success. Inherency and obviousness are entirely different concepts. *In re Spormann*, 53 CCPA 1375, 363 F.2d 444, 150 USPQ 449 (1966); *In re Adams*, 53 CCPA 996, 356 F.2d 998, 148 USPQ 742 (1966).

The board cited the indication in both Pengilly and Munro that their processes led to rapid reaction time and concluded that improved reaction time would be

expected if elements of those processes were combined. The evidence of record establishes, however, that reaction times of both prior processes lengthen as the processes are scaled up.

The board held the view that Munro's teaching of higher pressures to increase reaction rate would have provided an obvious solution to the problem Rinehart encountered in scaling up the process of Pengilly. But Rinehart's problem was not the need for increased reaction rate. It was, as the evidence established, the existence of lumps of frozen polymer. That problem is nowhere alluded to in either Pengilly or Munro, and of course no suggestion of a solution appears in either reference.

Moreover, Pengilly suggested that superatmospheric pressure [**18] was productive of certain disadvantages, particularly the need for use of a "large excess" of glycol. The use of superatmospheric pressure in a direct esterification process was referred to in other prior patents of record. With the exception of Munro, however, each such reference cited disadvantages of its use or an inability to find it workable. Munro's disclosure of superatmospheric pressure is rendered an abstraction with respect to appellant's problem by Munro's indication of the same excess glycol requirement when a large scale operation is contemplated. Munro employs a large excess of glycol (a ratio of glycol to acid of 3:1) in his example 5, the only example devoted to larger scale production. Rinehart's large scale production process is limited to a substantially equimolar ratio of glycol to acid. In view of all of the evidence, we cannot agree that Munro would suggest to one skilled in the art the use of superatmospheric pressure to solve the problem of scaling up the process of Pengilly.

Similarly, we find no suggestion in Pengilly or in Munro that Pengilly's preformed ester be employed in Munro's process to overcome the problems encountered in scaling up the process [**19] of Munro. Munro, as co-inventor with Lewis in earlier British Patent No. 776,282, was familiar with the use of a preformed polyester in direct esterification, yet neither Munro nor his co-inventor Maclean suggested its use with superatmospheric pressure in the cited reference. We find that the Munro patent contains its own solution to large scale operation, i.e., the use of excess glycol referred to above. That solution is not employed by appellant.

Absence of any suggestion in either Pengilly or Munro that features of the process of one should be combined with features of the other to achieve the commercial scale production of which neither is capable requires a holding that the rejection herein was improper. *In re Avery*, 518 F.2d 1228, 186 USPQ 161 (CCPA 1975). In view of that holding, it is unnecessary to consider Rinehart's allegations of commercial success

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and satisfaction of long-felt need.

REVERSED

The decision of the board is reversed.

« up

927 F.2d 1200

59 USLW 2575, 18 U.S.P.Q.2d 1016

AMGEN, INC., Plaintiff/Cross-Appellant,
v.CHUGAI PHARMACEUTICAL CO., LTD., and Genetics Institute,
Inc., Defendants-Appellants.

Nos. 90-1273, 90-1275.

**United States Court of Appeals,
Federal Circuit.***March 5, 1991.
Suggestion for Rehearing In Banc
Declined May 20, 1991.*

Edward M. O'Toole, Marshall, O'Toole, Gerstein, Murray & Bicknall, Chicago, Ill., argued, for plaintiff/cross-appellant. With him on the brief were Michael F. Borun, Richard A. Schnurr and Christine A. Dudzik. Also on the brief were Steven M. Odre and Robert D. Weist, Amgen, Inc., Thousand Oaks, Cal., of counsel.

Kurt E. Richter, Morgan & Finnegan, New York City, and William F. Lee, Hale & Dorr, Boston, Mass., argued for defendants-appellants. Of counsel were Eugene Moroz, Michael P. Dougherty and William S. Feiler, Morgan & Finnegan, New York City.

Before MARKEY, LOURIE and CLEVENGER, Circuit Judges.

LOURIE, Circuit Judge.

1 This appeal and cross appeal are from the March 4, 1990, judgment of the United States District Court for the District of Massachusetts, Amgen, Inc. v. Chugai Pharmaceutical Co., 13 USPQ2d 1737, 1989 WL 169006 (1990), and involve issues of patent validity, infringement, and inequitable conduct with respect to two patents: U.S. Patent 4,703,008 ('008), owned by Kirin-Amgen Inc. (Amgen), and U.S. Patent 4,677,195 ('195), owned by Genetics Institute, Inc. (GI).

2 Chugai Pharmaceutical Co., Ltd. (Chugai) and Genetics Institute, Inc. (collectively defendants) assert on appeal that the district court erred in holding that: 1) Amgen's '008 patent is not invalid under 35 U.S.C. Secs. 102(g) and 103; 2) the '008 patent is enforceable; 3) the failure of Amgen to deposit the best mode host cells was not a violation of the best mode requirement under 35 U.S.C. Sec. 112; and 4) claims 4 and 6 of GI's '195 patent are invalid for indefiniteness under 35 U.S.C. Sec. 112.

3 On cross appeal, Amgen challenges the district court's holdings that: 1) claims 1 and 3 of the '195 patent are enabled; 2) the '195 patent is enforceable; 3) this is not an exceptional case warranting an award of attorney fees to Amgen; and 4) claims 7, 8, 23-27 and 29 of the '008 patent are not enabled by the specification.

4 We affirm the district court's holdings in all respects, except that we reverse the court's ruling that claims 1 and 3 of the '195 patent are enabled. We also vacate that part of the district court's judgment relating to infringement of those claims.

« up BACKGROUND¹

5 Erythropoietin (EPO) is a protein consisting of 165 amino acids which stimulates the production of red blood cells. It is therefore a useful therapeutic agent in the treatment of anemias or blood disorders characterized by low or defective bone marrow production of red blood cells.

6 The preparation of EPO products generally has been accomplished through the concentration and purification of urine from both healthy individuals and those exhibiting high EPO levels. A new technique for producing EPO is recombinant DNA technology in which EPO is produced from cell cultures into which genetically-engineered vectors containing the EPO gene have been introduced. The production of EPO by recombinant technology involves expressing an EPO gene through the same processes that occur in a natural cell.

THE PATENTS

7 On June 30, 1987, the United States Patent and Trademark Office (PTO) issued to Dr. Rodney Hewick U.S. Patent 4,677,195, entitled "Method for the Purification of Erythropoietin and Erythropoietin Compositions" (the '195 patent). The patent claims both homogeneous EPO and compositions thereof and a method for purifying human EPO using reverse phase high performance liquid chromatography. The method claims are not before us. The relevant claims of the '195 patent are:

8 1. Homogeneous erythropoietin characterized by a molecular weight of about 34,000 daltons on SDS PAGE, movement as a single peak on reverse phase high performance liquid chromatography and a specific activity of at least 160,000 IU per absorbance unit at 280 nanometers.

9 * * * * *

10 3. A pharmaceutical composition for the treatment of anemia comprising a therapeutically effective amount of the homogeneous erythropoietin of claim 1 in a pharmaceutically acceptable vehicle.

11 4. Homogeneous erythropoietin characterized by a molecular weight of about 34,000 daltons on SDS PAGE, movement as a single peak on reverse phase high performance liquid chromatography and a specific activity of at least about 160,000 IU per absorbance unit at 280 nanometers.

12 * * * * *

13 6. A pharmaceutical composition for the treatment of anemia comprising a therapeutically effective amount of the homogeneous erythropoietin of claim 4 in a pharmaceutically acceptable vehicle.

14 Dr. Hewick assigned the patent to GI.

15 The other patent in this litigation is U.S. Patent 4,703,008, entitled "DNA Sequences Encoding Erythropoietin" (the '008 patent), issued on October 27, 1987, to Dr. Fu-Kuen Lin, an employee of Amgen. The claims of the '008 patent cover purified and isolated DNA sequences encoding erythropoietin and host cells transformed or transfected with a DNA sequence. The relevant claims are as follows:

16 2. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding human erythropoietin.

« up * * * * *

18 4. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to claim 1, 2 or 3 in a manner allowing the host cell to express erythropoietin.

19 * * * * *

20 6. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to claim 5.

21 7. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of that of erythropoietin to allow possession of the biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells, and to increase hemoglobin synthesis or iron uptake.

22 8. A cDNA sequence according to claim 7.

23 * * * * *

24 23. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to claim 7, 8, or 11 in a manner allowing the host cell to express said polypeptide.

25 24. A transformed or transfected host cell according to claim 23 which host cell is capable of glycosylating said polypeptide.

26 25. A transformed or transfected mammalian host cell according to claim 24.

27 26. A transformed or transfected COS cell according to claim 25.

28 27. A transformed or transfected CHO cell according to claim 25.

29 * * * * *

30 29. A procaryotic host cell stably transformed or transfected with a DNA vector according to claim 28.

PROCEDURAL HISTORY

31 On October 27, 1987, the same day that the '008 patent was issued, Amgen filed suit against Chugai and GI. It alleged that GI infringed the '008 patent by the production of recombinant EPO (rEPO) and by use of transformed mammalian host cells containing vectors with DNA coding for the production of human EPO, and that Chugai, as a result of a collaborative relationship with GI, had induced and/or contributed to the direct infringement of the '008 patent by GI. Amgen further sought a declaration that GI's '195 patent is invalid under 35 U.S.C. Secs. 102, 103, and 112, or, in the alternative, that Amgen does not infringe the claims of the '195 patent, and a declaration that GI and Chugai's future activities in the production and sale of rEPO will infringe the '008 patent.²

32 GI and Chugai answered and counterclaimed, asserting several affirmative defenses, including invalidity under 35 U.S.C. Secs. 101, 102, 103, and 112; non-infringement; failure to make deposits at a public depository of biological materials allegedly necessary for enabling the best mode of practicing the invention; and unenforceability of the patent because of Amgen's alleged inequitable conduct before the PTO. GI also counterclaimed,

« up leging that Amgen infringed the '195 patent, asserting unfair competition, and seeking a declaratory judgment that the '008 patent was invalid and not infringed.

33 GI and Chugai then filed a joint motion for a partial summary judgment that Amgen infringed the claims of the '195 patent. Chugai also filed its own motion for summary judgment. On February 24, 1988, the district court granted GI's and Chugai's motion for partial summary judgment and, on January 31, 1989, the court granted Chugai's motion for partial summary judgment only to the extent of ruling that the '008 patent does not contain a process claim, an issue that is not now before us.

34 In response to Amgen's motion for a preliminary injunction, the district court, on February 7, 1989, issued an order finding that "Amgen had shown a reasonable likelihood of success on the merits of the validity of its patent; that it would suffer irreparable injury due to the needs of an incipient market and the attendant burdens on a new company; ..." and that, as to the public interest, "recombinant EPO is an extraordinarily valuable medicine that promises marked relief from renal failure." Because of this public interest finding, the court determined that it would not enter an order to delay or prevent production or shipping of EPO, but would require the defendant GI to place with the court all profits from the sale of EPO.

35 In order to expedite trial, the parties consented to trial before a magistrate. The judge entered judgment upon findings of fact and conclusions of law set forth by the magistrate. With respect to Amgen's '008 patent, the court held that claims 2, 4, and 6 are valid, enforceable and have been infringed by GI; that infringement was not willful; that claims 7, 8, 23-27, and 29 are invalid for lack of enablement under 35 U.S.C. Sec. 112 but, if valid, were infringed by GI; that the '008 patent does not contain a process claim; and that Chugai has not infringed, contributorily infringed, or induced infringement of any claim of the '008 patent. The court also dismissed Amgen's complaint against Chugai.

36 With respect to GI's '195 patent, the court concluded that claims 1 and 3 are valid, enforceable, and have been infringed by Amgen; that Amgen has not infringed claims 2 and 5; that Amgen's infringement was not willful; and that claims 4 and 6 are invalid for indefiniteness under 35 U.S.C. Sec. 112, but, if valid, were infringed by Amgen. The court also concluded that Amgen did not misuse the '008 patent and that this was not an "exceptional" case under 35 U.S.C. Sec. 285.

DISCUSSION

I. AMGEN's '008 PATENT (Lin)

37 A. Alleged prior invention under 35 U.S.C. Sec. 102(g)

38 The first issue we review is whether the district court erred in finding that the claims directed to a purified and isolated DNA sequence encoding human EPO were not invalidated by the work of GI's Dr. Fritsch. Section 102(g) provides in relevant part that:

39 A person is entitled to a patent unless--

40 (g) before the applicant's invention thereof the invention was made ... by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other.

41 Defendants assert error in the district court's legal conclusion that in this case Lin's

« up Conception occurred simultaneously with reduction to practice. See e.g., Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1376, 231 USPQ 81, 87 (Fed.Cir.1986), cert. denied, 480 U.S. 947, 107 S.Ct. 1606, 94 L.Ed.2d 792 (1987). They claim that Fritsch was first to conceive a probing strategy of using two sets of fully-degenerate cDNA probes of two different regions of the EPO gene to screen a gDNA library, which was the strategy which the district court found eventually resulted in the successful identification and isolation of the EPO gene. Defendants further claim that Fritsch conceived this strategy in 1981, was diligent until he reduced the invention to practice in May of 1984, and thus should be held to be a Sec. 102(g) prior inventor over Lin, who reduced the invention to practice in September of 1983.

42 Conception is the "formation in the mind of the inventor, of a definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in practice." Hybritech, 802 F.2d at 1376, 231 USPQ at 87 (citing 1 Robinson on Patents 532 (1890)); Coleman v. Dines, 754 F.2d 353, 359, 224 USPQ 857, 862 (Fed.Cir.1985) (citing Gunter v. Stream, 573 F.2d 77, 80, 197 USPQ 482, 484 (CCPA 1978)). Conception requires both the idea of the invention's structure and possession of an operative method of making it. Oka v. Youssefeyeh, 849 F.2d 581, 583, 7 USPQ2d 1169, 1171 (Fed.Cir.1988).

43 In some instances, an inventor is unable to establish a conception until he has reduced the invention to practice through a successful experiment. This situation results in a simultaneous conception and reduction to practice. See 3 D. Chisum, Patents Sec. 10.04 (1990). We agree with the district court that that is what occurred in this case.

44 The invention recited in claim 2 is a "purified and isolated DNA sequence" encoding human EPO. The structure of this DNA sequence was unknown until 1983, when the gene was cloned by Lin; Fritsch was unaware of it until 1984. As Dr. Sadler, an expert for GI, testified in his deposition: "You have to clone it first to get the sequence." In order to design a set of degenerate probes, one of which will hybridize with a particular gene, the amino acid sequence, or a portion thereof, of the protein of interest must be known. Prior to 1983, the amino acid sequence for EPO was uncertain, and in some positions the sequence envisioned was incorrect. Thus, until Fritsch had a complete mental conception of a purified and isolated DNA sequence encoding EPO and a method for its preparation, in which the precise identity of the sequence is envisioned, or in terms of other characteristics sufficient to distinguish it from other genes, all he had was an objective to make an invention which he could not then adequately describe or define.

45 A gene is a chemical compound, albeit a complex one, and it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it. See Oka, 849 F.2d at 583, 7 USPQ2d at 1171. Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. It is not sufficient to define it solely by its principal biological property, e.g., encoding human erythropoietin, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property. We hold that when an inventor is unable to envision the detailed constitution of a gene so as to distinguish it from other materials, as well as a method for obtaining it, conception has not been achieved until reduction to practice has occurred, i.e., until after the gene has been isolated.

46 Fritsch had a goal of obtaining the isolated EPO gene, whatever its identity, and even had an idea of a possible method of obtaining it, but he did not conceive a purified and isolated DNA sequence encoding EPO and a viable method for obtaining it until after Lin.

« up : is important to recognize that neither Fritsch nor Lin invented EPO or the EPO gene. The subject matter of claim 2 was the novel purified and isolated sequence which codes for EPO, and neither Fritsch nor Lin knew the structure or physical characteristics of it and had a viable method of obtaining that subject matter until it was actually obtained and characterized.

47 Defendants further argue that because the trial court found that the probing and screening method employed by Lin is what distinguished the invention of the '008 patent over the prior art, Fritsch's strategy in 1981 had priority over Lin's use of that strategy. We disagree. The trial court found that Fritsch's alleged conception in 1981 of an approach that might result in cloning the gene was mere speculation. Conception of a generalized approach for screening a DNA library that might be used to identify and clone the EPO gene of then unknown constitution is not conception of a "purified and isolated DNA sequence" encoding human EPO. It is not "a definite and permanent idea of the complete and operative invention." Fritsch's conception of a process had to be sufficiently specific that one skilled in the relevant art would succeed in cloning the EPO gene. See Coleman, 754 F.2d at 359, 224 USPQ at 862. Clearly, he did not have that conception because he did not know the structure of EPO or the EPO gene.

48 The record indicates that several companies, as well as Amgen and GI, were unsuccessful using Fritsch's approach. As the trial court correctly summarized:

49 Given the utter lack of experience in probing genomic libraries with fully degenerate probes and the crudeness of the techniques available in 1981, it would have been mere speculation or at most a probable deduction from facts then known by Dr. Fritsch that his generalized approach would result in cloning the EPO gene.

50 13 USPQ2d at 1760. As expert testimony from both sides indicated, success in cloning the EPO gene was not assured until the gene was in fact isolated and its sequence known. Based on the uncertainties of the method and lack of information concerning the amino acid sequence of the EPO protein, the trial court was correct in concluding that neither party had an adequate conception of the DNA sequence until reduction to practice had been achieved; Lin was first to accomplish that goal.

51 Defendants also argue that the court failed to consider that 1983, just prior to Lin's conception, was the relevant time for determining the completeness of Fritsch's conception, not 1981. However, the record shows that the court did consider what occurred in 1983. Moreover, Fritsch had no more of a conception in 1983 than he did in 1981, because he did not then know the sequence of the gene encoding EPO.

52 B. Alleged obviousness of the inventions of claims 2, 4, and 6

53 Claim 2, as noted above, recites a purified and isolated DNA sequence, and claims 4 and 6 are directed to host cells transformed with such a DNA sequence. The district court determined that claims 2, 4, and 6 are not invalid under 35 U.S.C. Sec. 103, concluding that the unique probing and screening method employed by Lin in isolating the EPO gene and the extensive effort required to employ that method made the invention nonobvious over the prior art.³

54 Obviousness under Section 103 is a question of law. *Panduit Corp. v. Dennison Mfg. Co.*, 810 F.2d 1561, 1568, 1 USPQ2d 1593, 1597 (Fed.Cir.), cert. denied, 481 U.S. 1052, 107 S.Ct. 2187, 95 L.Ed.2d 843 (1987). The district court stated that one must inquire whether the prior art would have suggested to one of ordinary skill in the art that Lin's probing and screening method should be carried out and would have a reasonable expectation of success, viewed in light of the prior art. See *In re Dow Chemical Co.*, 837 F.2d 469, 473, 5

« up [SPQ2d 1529, 1531 (Fed.Cir.1988). "Both the suggestion and the expectation of success must be founded in the prior art, not in applicant's disclosure." Id.

55 The district court specifically found that, as of 1983, none of the prior art references "suggest[s] that the probing strategy of using two fully-redundant [sic] sets of probes, of relatively high degeneracy [sic], to screen a human genomic library would be likely to succeed in pulling out the gene of interest."⁴ 13 USPQ2d at 1768. While it found that defendants had shown that these procedures were "obvious to try," the references did not show that there was a reasonable expectation of success. See *In re O'Farrell*, 853 F.2d 894, 903-04, 7 USPQ2d 1673, 1680-81 (Fed.Cir.1988).

56 Defendants challenge the district court's determination, arguing that, as of September 1983, one of ordinary skill in the art would have had a reasonable expectation of success in screening a gDNA library by Lin's method in order to obtain EPO. We agree with the district court's conclusion, which was supported by convincing testimony. One witness, Dr. Davies of Biogen, another biotechnology company that had worked on EPO, stated that he could not say whether Biogen scientists would have succeeded in isolating the EPO gene if Biogen had the EPO fragments that were available to Lin in 1983. Dr. Wall, a professor at UCLA, testified that it would have been "difficult" to find the gene in 1983, and that there would have been no more than a fifty percent chance of success. He said, "you couldn't be certain where in the genomic DNA your probe might fall." The court found that no one had successfully screened a genomic library using fully-degenerate probes of such high redundancy as the probes used by Lin. In the face of this and other evidence on both sides of the issue, it concluded that defendants had not shown by clear and convincing evidence that the procedures used by Lin would have been obvious in September 1983. We are not persuaded that the court erred in its decision.

57 Defendants assert that whether or not it would have been obvious to isolate the human EPO gene from a gDNA library with fully-degenerate probes is immaterial because it was obvious to use the already known monkey EPO gene as a probe. Defendants point out that, in the early 1980s, Biogen did significant work with an EPO cDNA obtained from a baboon, and that they used it as a probe to hybridize with the corresponding gene in a human gDNA library. However, this technique did not succeed until after Lin isolated the EPO gene with his fully-degenerate set of probes.

58 To support its obviousness assertion, defendants rely upon the testimony of their expert, Dr. Flavell, who testified that the overall homology of baboon DNA and human DNA was "roughly 90 percent". While this testimony indicates that it might have been feasible, perhaps obvious to try, to successfully probe a human gDNA library with a monkey cDNA probe, it does not indicate that the gene could have been identified and isolated with a reasonable likelihood of success. Neither the DNA nucleotide sequence of the human EPO gene nor its exact degree of homology with the monkey EPO gene was known at the time.

59 Indeed, the district court found that Lin was unsuccessful at probing a human gDNA library with monkey cDNA until after he had isolated the EPO gene by using the fully-degenerate probes. Based on the evidence in the record, the district court found there was no reasonable expectation of success in obtaining the EPO gene by the method that Lin eventually used. While the idea of using the monkey gene to probe for a homologous human gene may have been obvious to try, the realization of that idea would not have been obvious. There were many pitfalls. Hindsight is not a justifiable basis on which to find that ultimate achievement of a long sought and difficult scientific goal was obvious. The district court thoroughly examined the evidence and the testimony. We see no error in its result. Moreover, if the DNA sequence was not obvious, host cells containing such

« up sequence, as claimed in claims 4 and 6, could not have been obvious. We conclude that the district court did not err in holding that the claims of the patent are not invalid under Section 103.

60 C. Best Mode

61 Defendants argue that the district court erred in failing to hold the '008 patent invalid under 35 U.S.C. Sec. 112, asserting that Lin failed to disclose the best mammalian host cells known to him as of November 30, 1984, the date he filed his fourth patent application.

62 The district court found that the "best mode" of practicing the claimed invention was by use of a specific genetically-heterogeneous strain of Chinese hamster ovary (CHO) cells, which produced EPO at a rate greater than that of other cells. It further found that this strain was disclosed in Example 10 and that Lin knew of no better mode. GI argues that Lin's best mode was not adequately disclosed in Example 10 because one skilled in the art could not duplicate Lin's best mode without his having first deposited a sample of the specific cells in a public depository. The issue before us therefore is whether the district court erred in concluding that Example 10 of the '008 patent satisfied the best mode requirement as to the invention of the challenged claims⁵ and that a deposit of the preferred CHO cells was not necessary.

63 A determination whether the best mode requirement is satisfied is a question of fact, DeGeorge v. Bernier, 768 F.2d 1318, 1324, 226 USPQ 758, 763 (Fed.Cir.1985); we therefore review the district court's finding under a clearly erroneous standard.

64 35 U.S.C. Sec. 112 provides in relevant part:

65 The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

66 (Emphasis added).

67 This court has recently discussed the best mode requirement, pointing out that its analysis has two components. Chemcast Corp. v. Arco Indus. Corp., 913 F.2d 923, 927, 16 USPQ2d 1033, 1036 (Fed.Cir.1990). The first is a subjective one, asking whether, at the time the inventor filed his patent application, he contemplated a best mode of practicing his invention. If he did, the second inquiry is whether his disclosure is adequate to enable one skilled in the art to practice the best mode or, in other words, whether the best mode has been concealed from the public. The best mode requirement thus is intended to ensure that a patent applicant plays "fair and square" with the patent system. It is a requirement that the quid pro quo of the patent grant be satisfied. One must not receive the right to exclude others unless at the time of filing he has provided an adequate disclosure of the best mode known to him of carrying out his invention. Our case law has interpreted the best mode requirement to mean that there must be no concealment of a mode known by the inventor to be better than that which is disclosed. Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1384-85, 231 USPQ 81, 94 (Fed.Cir.1986), cert. denied, 480 U.S. 947, 107 S.Ct. 1606, 94 L.Ed.2d 792 (1987). Section 282 imposes on those attempting to prove invalidity the burden of proof. We agree that the district court did not err in finding that defendants have not met their burden of proving a best mode violation.

« up As noted above, the district court found that the best mode of making the CHO cells . . . as set forth in Example 10. As the district court stated, while it was not clear which of two possible strains Lin considered to be the best, the cell strain subjected to 1000 nanomolar MTX (methotrexate) or that subjected to 100 nanomolar MTX, the best mode was disclosed because both were disclosed.⁶ Defendants argue that this disclosure is not enough, that a deposit of the cells was required.

68 Defendants contend that "[i]n the field of living materials such as microorganisms and cell cultures," we should require a biological deposit so that the public has access to exactly the best mode contemplated by the inventor. This presents us with a question of first impression concerning the best mode requirement for patents involving novel genetically-engineered biological subject matter.

69 For many years, it has been customary for patent applicants to place microorganism samples in a public depository when such a sample is necessary to carry out a claimed invention. This practice arose out of the development of antibiotics, when microorganisms obtained from soil samples uniquely synthesized antibiotics which could not be readily prepared chemically or otherwise. *In re Argoudelis*, 434 F.2d 1390, 168 USPQ 99 (CCPA 1970). Such a deposit has been considered adequate to satisfy the enablement requirement of 35 U.S.C. Sec. 112, when a written description alone would not place the invention in the hands of the public and physical possession of a unique biological material is required. See, e.g., *In re Wands*, 858 F.2d 731, 735-36, 8 USPQ2d 1400, 1403 (Fed.Cir.1988) ("Where an invention depends on the use of living materials ... it may be impossible to enable the public to make the invention (i.e., to obtain these living materials) solely by means of written disclosure."); *In re Lundak*, 773 F.2d 1216, 1220, 227 USPQ 90, 93 (Fed.Cir.1985) ("When an invention relates to a new biological material, the material may not be reproducible even when detailed procedures and a complete taxonomic description are included in the specification."); see generally *Hampar, Patenting of Recombinant DNA Technology: The Deposit Requirement*, 67 J. Pat. & Trademark Off. Soc'y 569, 607 (1985) ("The deposit requirement is a nonstatutory mechanism for ensuring compliance with the 'enabling' provision under 35 U.S.C. Sec. 112.").

70 The district court found that the claims at issue require the use of biological materials that were capable of being prepared in the laboratory from readily available biological cells, using the description in Example 10. The court also found that there were no starting materials that were not publicly available, that were not described, or that required undue experimentation for their preparation in order to carry out the best mode. The court noted that Lin testified that the isolation of the preferred strain was a "routine limited dilution cloning procedure[]" well known in the art. Dr. Simonsen, GI's own expert, testified that the disclosed procedures were "standard" and that:

71 with the vectors and the sequences shown in Example 10, I have no doubt that someone eventually could reproduce--well, could generate cell lines [sic, strains] making some level of EPO, and they could be better, they could be worse in terms of EPO production.

72 The district court relied on this testimony, and, upon review, we agree with its determination. The testimony accurately reflects that the invention, as it relates to the best mode host cells, could be practiced by one skilled in the art following Example 10. Thus, the best mode was disclosed and it was adequately enabled.

73 These materials are therefore not analogous to the biological cells obtained from unique soil samples. When a biological sample required for the practice of an invention is obtained from nature, the invention may be incapable of being practiced without access

« up) that organism. Hence the deposit is required in that case. On the other hand, when, as in the case here, the organism is created by insertion of genetic material into a cell obtained from generally available sources, then all that is required is a description of the best mode and an adequate description of the means of carrying out the invention, not deposit of the cells. If the cells can be prepared without undue experimentation from known materials, based on the description in the patent specification, a deposit is not required. See *Feldman v. Aunstrup*, 517 F.2d 1351, 1354, 186 USPQ 108, 111 (CCPA 1975), ("No problem exists when the microorganisms used are known and readily available to the public."), cert. denied, 424 U.S. 912, 96 S.Ct. 1109, 47 L.Ed.2d 316 (1976). Since the court found that that is the case here, we therefore hold that there is no failure to comply with the best mode requirement for lack of a deposit of the CHO cells, when the best mode of preparing the cells has been disclosed and the best mode cells have been enabled, i.e., they can be prepared by one skilled in the art from known materials using the description in the specification.

74 Defendants also contend that the examiner's rejection of the application that matured into the '008 patent for failure to make a publicly accessible biological deposit supports its argument. U.S. Patent Application Serial No. 675,298, Prosecution History at 179 (First Rejection July 3, 1986). However, that rejection was withdrawn after an oral interview and a written argument that the invention did not require a deposit. *Id.* at 208.

75 We also note that the PTO has recently prescribed guidelines concerning the deposit of biological materials. See 37 C.F.R. Sec. 1.802(b) (1990) (biological material need not be deposited "if it is known and readily available to the public or can be made or isolated without undue experimentation"). The PTO, in response to a question as to whether the deposit requirement is applicable to the best mode requirement, as distinct from enablement, said:

76 The best mode requirement is a safeguard against the possible selfish desire on the part of some people to obtain patent protection without making a full disclosure. The requirement does not permit an inventor to disclose only what is known to be the second-best embodiment, retaining the best.... The fundamental issue that should be addressed is whether there was evidence to show that the quality of an applicant's best mode disclosure is so poor as to effectively result in concealment. *In re Sherwood*, 615 F.2d 809, 204 USPQ 537 (CCPA 1980). If a deposit is the only way to comply with the best mode requirement then the deposit must be made.

52 Fed.Reg. 34080, 34086 (Sept. 8, 1987).⁷

77 We see no inconsistency between the district court's decision, which we affirm here, and these guidelines.

78 Defendants also assert that the record shows that scientists were unable to duplicate Lin's genetically-heterogeneous best mode cell strain. However, we have long held that the issue is whether the disclosure is "adequate," not that an exact duplication is necessary. Indeed, the district court stated that

79 [t]he testimony is clear that no scientist could ever duplicate exactly the best mode used by Amgen, but that those of ordinary skill in the art could produce mammalian host cell strains or lines with similar levels of production identified in Example 10.

80 13 USPQ2d at 1774. What is required is an adequate disclosure of the best mode, not a guarantee that every aspect of the specification be precisely and universally reproducible. See *In re Gay*, 309 F.2d 769, 773, 135 USPQ 311, 316, 50 CCPA 725 (1962).

« up Defendants finally argue that Lin's failure to deposit the transfected cells notwithstanding the fact that he was willing to deposit essentially worthless cell material was evidence of deliberate concealment. We have already stated that deposit of the host cells containing the rEPO gene was not necessary to satisfy the best mode requirement of Section 112. The best mode was disclosed and a deposit was not necessary to carry it out. Therefore, the fact that some cells were deposited, but not others, is irrelevant.

D. Enablement of claims 7, 8, 23-27, and 29

82 Amgen argues that the district court's holding that GI "provided clear and convincing evidence that the patent specification is insufficient to enable one of ordinary skill in the art to make and use the invention claimed in claim 7 of the '008 patent without undue experimentation" constituted legal error. 13 USPQ2d at 1776. Amgen specifically argues that the district court erred because it "did not properly address the factors which this court has held must be considered in determining lack of enablement based on assertion of undue experimentation," citing this court's decision in *In re Wands*, 858 F.2d at 737, 8 USPQ2d at 1404.

83 Claim 7 is a generic claim, covering all possible DNA sequences that will encode any polypeptide having an amino acid sequence "sufficiently duplicative" of EPO to possess the property of increasing production of red blood cells. As claims 8, 23-27, and 29, dependent on claim 7, are not separately argued, and are of similar scope, they stand or fall with claim 7. See *In re Dillon*, 919 F.2d 688, 692, 16 USPQ2d 1897, 1900 (Fed.Cir.1990) (in banc).

84 Whether a claimed invention is enabled under 35 U.S.C. Sec. 112 is a question of law, which we review de novo. *Moleculon Research Corp. v. CBS, Inc.*, 793 F.2d 1261, 1268, 229 USPQ 805, 811 (Fed.Cir.1986), cert. denied, 479 U.S. 1030, 107 S.Ct. 875, 93 L.Ed.2d 829 (1987). "To be enabling under Sec. 112, a patent must contain a description that enables one skilled in the art to make and use the claimed invention." *Atlas Powder Co. v. E.I. duPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed.Cir.1984).

85 That some experimentation is necessary does not constitute a lack of enablement; the amount of experimentation, however, must not be unduly extensive. *Id.* The essential question here is whether the scope of enablement of claim 7 is as broad as the scope of the claim. See generally *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970); 2 D. Chisum, *Patents* Sec. 7.03[b] (1990).

86 The specification of the '008 patent provides that:

87 one may readily design and manufacture genes coding for microbial expression of polypeptides having primary conformations which differ from that herein specified for mature EPO in terms of the identity or location of one or more residues (e.g., substitutions, terminal and intermediate additions and deletions).

88 * * * * *

89 DNA sequences provided by the present invention are thus seen to comprehend all DNA sequences suitable for use in securing expression in a prokaryotic or eukaryotic host cell of a polypeptide product having at least a part of the primary structural conformation and one or more of the biological properties of erythropoietin, and selected from among: (a) the DNA sequences set out in FIGS. 5 and 6; (b) DNA sequences which hybridize to the DNA sequences defined in (a) or fragments thereof; and (c) DNA sequences which, but for the degeneracy of the genetic code, would hybridize to the DNA sequences defined in (a) and (b).

« up The district court found that over 3,600 different EPO analogs can be made by substituting at only a single amino acid position, and over a million different analogs can be made by substituting three amino acids. The patent indicates that it embraces means for preparation of "numerous" polypeptide analogs of EPO. Thus, the number of claimed DNA encoding sequences that can produce an EPO-like product is potentially enormous.

91 In a deposition, Dr. Elliott, who was head of Amgen's EPO analog program, testified that he did not know whether the fifty to eighty EPO analogs Amgen had made "had the biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells, and to increase hemoglobin synthesis or iron uptake." Based on this evidence, the trial court concluded that "defendants had provided clear and convincing evidence that the patent specification is insufficient to enable one of ordinary skill in the art to make and use the invention claimed in claim 7 of the '008 patent without undue experimentation." 13 USPQ at 1776. In making this determination, the court relied in particular on the lack of predictability in the art, as demonstrated by the testimony of both Dr. Goldwasser, another scientist who worked on procedures for purifying urinary EPO (uEPO), and Dr. Elliott. After five years of experimentation, the court noted, "Amgen is still unable to specify which analogs have the biological properties set forth in claim 7." Id.

92 We believe the trial court arrived at the correct decision, although for the wrong reason. By focusing on the biological properties of the EPO analogs, it failed to consider the enablement of the DNA sequence analogs, which are the subject of claim 7. Moreover, it is not necessary that a patent applicant test all the embodiments of his invention, *In re Angstadt*, 537 F.2d 498, 502, 190 USPQ 214, 218 (CCPA 1976); what is necessary is that he provide a disclosure sufficient to enable one skilled in the art to carry out the invention commensurate with the scope of his claims. For DNA sequences, that means disclosing how to make and use enough sequences to justify grant of the claims sought. Amgen has not done that here. In addition, it is not necessary that a court review all the Wands factors to find a disclosure enabling. They are illustrative, not mandatory. What is relevant depends on the facts, and the facts here are that Amgen has not enabled preparation of DNA sequences sufficient to support its all-encompassing claims.

93 It is well established that a patent applicant is entitled to claim his invention generically, when he describes it sufficiently to meet the requirements of Section 112. See *Utter v. Hiraga*, 845 F.2d 993, 998, 6 USPQ2d 1709, 1714 (Fed.Cir.1988) ("A specification may, within the meaning of 35 U.S.C. Sec. 112 p 1, contain a written description of a broadly claimed invention without describing all species that claim encompasses."); *In re Robins*, 429 F.2d 452, 456-57, 166 USPQ 552, 555 (CCPA 1970) ("[R]epresentative samples are not required by the statute and are not an end in themselves."). Here, however, despite extensive statements in the specification concerning all the analogs of the EPO gene that can be made, there is little enabling disclosure of particular analogs and how to make them. Details for preparing only a few EPO analog genes are disclosed. Amgen argues that this is sufficient to support its claims; we disagree. This "disclosure" might well justify a generic claim encompassing these and similar analogs, but it represents inadequate support for Amgen's desire to claim all EPO gene analogs. There may be many other genetic sequences that code for EPO-type products. Amgen has told how to make and use only a few of them and is therefore not entitled to claim all of them.

94 In affirming the district court's invalidation of claims 7, 8, 23-27, and 29 under Section 112, we do not intend to imply that generic claims to genetic sequences cannot be valid where they are of a scope appropriate to the invention disclosed by an applicant. That is not the case here, where Amgen has claimed every possible analog of a gene containing about 4,000 nucleotides, with a disclosure only of how to make EPO and a very few

« up analogs.

95 The district court properly relied upon Fisher⁸ in making its decision. In that case, an applicant was attempting to claim an adrenocorticotropic hormone preparation containing a polypeptide having at least twenty-four amino acids of a specified sequence. Only a thirty-nine amino acid product was disclosed. The court found that applicant could not obtain claims that are insufficiently supported and hence not in compliance with the first paragraph of 35 U.S.C. Sec. 112. It stated:

96 Appellant's parent application, therefore, discloses no products, inherently or expressly, containing other than 39 amino acids, yet the claim includes all polypeptides, of the recited potency and purity, having at least 24 amino acids in the chain in the recited sequence. The parent specification does not enable one skilled in the art to make or obtain ACTHs with other than 39 amino acids in the chain, and there has been no showing that one of ordinary skill would have known how to make or obtain such other ACTHs without undue experimentation. As for appellant's conclusion that the 25th to 39th acids in the chain are unnecessary, it is one thing to make such a statement when persons skilled in the art are able to make or obtain ACTH having other than 39 amino acids; it is quite another thing when they are not able to do so. In the latter situation, the statement is in no way "enabling" and hence lends no further support for the broad claim. We conclude that appellant's parent application is insufficient to support a claim as broad as claim 4.

97 * * * * *

98 [Section 112] requires that the scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art.

99 Fisher, 427 F.2d at 836, 839, 166 USPQ at 21-22, 24.

100 Considering the structural complexity of the EPO gene, the manifold possibilities for change in its structure, with attendant uncertainty as to what utility will be possessed by these analogs, we consider that more is needed concerning identifying the various analogs that are within the scope of the claim, methods for making them, and structural requirements for producing compounds with EPO-like activity. It is not sufficient, having made the gene and a handful of analogs whose activity has not been clearly ascertained, to claim all possible genetic sequences that have EPO-like activity. Under the circumstances, we find no error in the court's conclusion that the generic DNA sequence claims are invalid under Section 112.

E. Inequitable Conduct

101 Defendants argue that the '008 patent claims are unenforceable as a result of an asserted misrepresentation of the number of probes Lin used for the monkey gene cloning described in Example 3 of his patent. Relying on the district court's finding that Lin had said that a "full set" mixture of 128 "EpV" probes⁹ was used for monkey cDNA screening, whereas only a 16-member "subset" of the EpV mixture was actually used, defendants argue that the court ought to have found that the representations were material.

102 The essential elements of proof of inequitable conduct include intent to deceive and materiality. After finding threshold levels of materiality and intent, the trial court must balance the two and determine, in its discretion, whether inequitable conduct has occurred. J.P. Stevens & Co. v. Lex Tex Ltd., Inc., 747 F.2d 1553, 1560, 223 USPQ 1089,

« up 392 (Fed.Cir.1984), cert. denied, 474 U.S. 822, 106 S.Ct. 73, 88 L.Ed.2d 60 (1985). While we review an ultimate conclusion of inequitable conduct under an abuse of discretion standard, Kingsdown Medical Consultants, Ltd. v. Hollister Inc., 863 F.2d 867, 876, 9 USPQ2d 1384, 1392 (Fed.Cir.1988) (in banc), cert. denied, 490 U.S. 1067, 109 S.Ct. 2068, 104 L.Ed.2d 633 (1989), the underlying factual threshold findings are reviewed under a clearly erroneous standard.

103 Lin set out to clone the EPO gene by more than one method, including using degenerate human probes and monkey probes. It is not disputed that he did isolate the human EPO gene from a genomic library using two different 128-member pools of probes made from fragments of the human EPO protein. Thereafter, he also attempted to use the human sequence probes to find the monkey EPO cDNA to be used later as a probe to hybridize with the human EPO gene. Example 3 of the '008 patent describes this work, indicating that the screening yielded seven positive clones. It also reports that a subset of the human EpV mixture was used for DNA sequencing work. When Lin published his monkey cDNA cloning work in a scientific journal, he also reported the use of 128 EpV probes to screen the monkey library. Lin screened the monkey library with the full mixture of 128 EpV probes and with one of eight subsets of probes which made up the full EpV mixture. In response to a question whether a subset of EpV probes was used in the first screening of the monkey cDNA library, Lin testified:

104 I don't know which we used, the subset first or used the full set first. I cannot recall exactly. It looks like the subset was first defining the number, yes.

105 This answer constituted the sole basis for the court's finding that, "[a]t trial, Lin admitted he only used a subset of the EpV 128 probes in screening the cDNA library." 13 USPQ2d at 1778.

106 We consider that the district court's finding of an "admission" of misrepresentation in Lin's testimony and its conclusion that GI "presented clear and convincing evidence of a misrepresentation" was clearly erroneous. That Lin did not recall whether he first screened the monkey cDNA library with a full set of probes or a subset of probes, and his answer that "it looks like" he used the subset, are certainly not clear admissions that he only used a subset. However, the district court was correct in concluding that, even if there had been an erroneous statement, it was not material because Lin succeeded in cloning the EPO gene first with his use of the fully-degenerate probes. Thus, his testimony does not provide clear and convincing evidence that he misrepresented to the PTO the number of probes used. He did use 128-member probes as well as a subset. Moreover, this evidence does not create an inference of an intent to mislead. The court properly concluded that there was no inequitable conduct in prosecuting the '008 patent.

II. GI's '195 PATENT (Hewick)

A. Enablement of claims 1 and 3

107 Amgen challenges the district court's determination that "the '195 patent enables a person of ordinary skill in the art to obtain homogeneous EPO [including rEPO and uEPO] from natural sources" having a mean in vivo specific activity of at least 160,000.¹⁰ 13 USPQ2d at 1794. Claims 1 and 3 contain the limitation that EPO have a specific activity of at least 160,000 IU/AU. The district court found, based upon expert testimony from both sides, that to those skilled in the art, in the absence of an express statement in the patent, the claims would be construed to refer to in vivo rather than in vitro specific activity. To support its challenge, Amgen asserts that the district court's determination is contradicted by GI's own bioassay data and by the district court's finding that "the '195 patent fails to enable the purification of rEPO." Amgen also asserts that the district court

« up tried in relying solely on an in vitro measure of specific activity, having initially construed the '195 claims as requiring an in vivo measure to avoid invalidity for indefiniteness.

108 35 U.S.C. Sec. 112 requires that an invention be described "in such full, clear, concise, and exact terms as to enable any person skilled in the art ... to make and use the same." We review a determination of enablement as a question of law. Moleculon Research Corp. v. CBS, Inc., 793 F.2d 1261, 1268, 229 USPQ 805, 811 (Fed.Cir.1986), cert. denied, 479 U.S. 1030, 107 S.Ct. 875, 93 L.Ed.2d 829 (1987).

109 We do not consider the court's finding that the assay measurement was an in vivo one to be erroneous in view of the testimony it heard. That being the case, the question is whether the court erred in concluding that the claims requiring 160,000 IU/AU by an in vivo measurement were enabled. We conclude that it did err.

110 Defendants have produced no evidence that it ever prepared EPO with a specific activity of at least 160,000 IU/AU in vivo using the disclosed methods. In its report to the FDA, GI stated that it had purified uEPO material "to homogeneity" by subjecting partially purified uEPO material to reverse phase high performance liquid chromatography (RP-HPLC), the technique taught by Hewick in the '195 patent. The district court found that GI reported to the FDA that the specific activity of uEPO, based on in vivo bioassays, was only 109,000 IU/AU.¹¹ GI originally arrived at the figure of 160,000 IU/AU by calculation, before it had the capacity to derive quantitative information from bioassays. Hewick subjected the EPO to RP-HPLC, the EPO having an actual value of 83,000 IU/AU. After weighing the chromatograph, he found that "at least fifty percent" of the area under the chromatograph curve was attributable to something other than EPO. He then doubled the 83,000, and arrived at a theoretical specific activity of "at least about 160,000 IU/AU." That procedure, while possibly valid as a means for estimating the specific activity of a pure sample, does not establish that GI had a workable method for actually obtaining the pure material that it claimed.

111 Moreover, the work of others shows that Hewick did not enable the preparation of uEPO having an in vivo specific activity of at least 160,000, as the claims required. Dr. Kawakita, a scientist at Kumamoto University in Japan, reported an in vivo specific activity of 101,000 IU/AU when using RP-HPLC according to Hewick's method. This is similar to the 109,000 value reported to the FDA by GI. Kawakita did report a value of 188,000, but did not follow the teachings in the '195 patent. Defendants also rely on the testimony of Fritsch that "I've also seen further data in Chugai's PLA indicating additional urinary EPO preparation that had activities of 190,000, I believe, units per absorbance unit." However, the document to which Fritsch referred was not offered into evidence by GI after Amgen objected to its introduction and is not before us.

112 Defendants argue that Dr. Kung's uEPO test result of 173,640 IU/AU in an in vitro test supports the enablement of its claims. Amgen argues that an in vivo test result would only have been 65 percent of the in vitro result and thus would not have met the 160,000 IU/AU limitation of the claims. The district court relied on Kung, despite the demonstrated disparity between the results of in vitro and in vivo testing.

113 It is not absolutely clear to us that, for uEPO, the in vivo specific activity is 65 percent of the in vitro specific activity. Nonetheless, Kung's measurement, being in vitro, does not demonstrate enablement of the claimed invention, and that fact means that the court erred in finding enablement. Added to this fact is the difference that exists between the in vivo results for rEPO and uEPO¹², and the other lack of support for the 160,000 limitation. Under these circumstances, we hold that the district court erred in accepting the in vitro data as support for claims containing what has been found to be an in vivo limitation.

« up In addition to the question of enablement regarding uEPO, the district court found that the only purification attempt on rEPO in the manner set out in the '195 patent failed to provide homogeneous EPO. The patent itself, in Example 2, discloses GI's purification efforts on rEPO and indicates that GI did not obtain purified rEPO. As the district court found, "[t]he patent does not contain any procedures ... for purifying rEPO to the point that RP-HPLC will be successful." 13 USPQ2d at 1758. Thus, the patent fails to enable purification of either rEPO or uEPO.¹³ See *In re Rainer*, 377 F.2d 1006, 1012, 153 USPQ 802, 807, 54 CCPA 1445 (1967) ("specification is evidence of its own inadequacy").

115 The burden of showing non-enablement is Amgen's, not GI's, but in the case of a challenged patent, when substantial discovery has occurred, and there is no credible evidence that the claimed purified material can be made by those skilled in the art by the disclosed process, and all evidence from both the inventor and his assignee and from third parties is to the contrary, we conclude that Amgen has met its burden to show that the claims have not been adequately enabled. We do not hold that one must always prove that a disclosed process operates effectively to produce a claimed product. But, under these circumstances, we conclude that the court erred in holding that claims 1 and 3 were properly enabled.

B. Indefiniteness of claims 4 and 6

116 The district court held claims 4 and 6 of the '195 patent invalid because their specific activity limitation of "at least about 160,000" was indefinite. Defendants challenge this holding, asserting that there is no evidence that claims 4 and 6 do not comply with the requirements of 35 U.S.C. Sec. 112.

117 The statute requires that "[t]he specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention." A decision as to whether a claim is invalid under this provision requires a determination whether those skilled in the art would understand what is claimed. See *Shatterproof Glass Corp. v. Libbey-Owens Ford Co.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed.Cir.1985) (Claims must "reasonably apprise those skilled in the art" as to their scope and be "as precise as the subject matter permits."). The district court found that "bioassays provide an imprecise form of measurement with a range of error" and that use of the term "about" 160,000 IU/AU, coupled with the range of error already inherent in the specific activity limitation, served neither to distinguish the invention over the close prior art (which described preparations of 120,000 IU/AU), nor to permit one to know what specific activity values below 160,000, if any, might constitute infringement. 13 USPQ2d at 1787. It found evidence of ambiguity in the fact that Chugai, GI's partner, itself questioned whether the specific activity value of 138,000 IU/AU for its own rEPO was within the claim coverage.

118 In prosecuting the '195 patent, GI disclosed to the examiner a publication by Miyake et al., which discloses a uEPO product having an in vivo specific activity of 128,620 IU/AU. When the examiner noticed this disclosure late in the prosecution, he rejected the '195 claims with a specific activity limitation of "at least 120,000" as anticipated by the Miyake et al. disclosure. It was only after the "at least 120,000" claims were cancelled that GI submitted the "at least about 160,000" claim language.

119 The court found the "addition of the word 'about' seems to constitute an effort to recapture ... a mean activity somewhere between 120,000, which the patent examiner found was anticipated by the prior art, and [the] 160,000 IU/AU" claims which were previously allowed. Because "the term 'about' 160,000 gives no hint as to which mean value between the Miyake et al. value of 128,620 and the mean specific activity level of 160,000 constitutes infringement," the court held the "at least about" claims to be invalid

« up or indefiniteness. 13 USPQ2d at 1787-88. This holding was further supported by the fact that nothing in the specification, prosecution history, or prior art provides any indication as to what range of specific activity is covered by the term "about," and by the fact that no expert testified as to a definite meaning for the term in the context of the prior art. In his testimony, Fritsch tried to define "about" 160,000, but he could only say that while "somewhere between 155[,000] might fit within that number," he had not "given a lot of direct considerations to that...."

120 When the meaning of claims is in doubt, especially when, as is the case here, there is close prior art, they are properly declared invalid. Standard Oil Co. v. American Cyanamid Co., 774 F.2d 448, 453, 227 USPQ 293, 297 (Fed.Cir.1985). We therefore affirm the district court's determination on this issue. We also note that, in view of our reversal of the district court's holding that claims 1 and 3 are valid, it is clear that claims 4 and 6 would also be invalid without the "about" limitation. In arriving at this conclusion, we caution that our holding that the term "about" renders indefinite claims 4 and 6 should not be understood as ruling out any and all uses of this term in patent claims. It may be acceptable in appropriate fact situations, e.g., W.L. Gore & Assocs., Inc. v. Garlock, Inc., 721 F.2d 1540, 1557, 220 USPQ 303, 316 (Fed.Cir.1983) ("use of 'stretching ... at a rate exceeding about 10% per second' in the claims is not indefinite"), even though it is not here.

C. Inequitable Conduct

121 The district court concluded that GI did not engage in inequitable conduct with respect to the '195 patent. Amgen challenges this holding, asserting, *inter alia*, that GI displayed an intent to mislead by withholding data showing *in vivo* specific activity of homogenous uEPO and withholding information on the range of error in EPO bioassays.

122 It is fundamental that to establish inequitable conduct, an intent to deceive is required. *RCA Corp. v. Data General Corp.*, 887 F.2d 1056, 1065, 12 USPQ2d 1449, 1456-57 (Fed.Cir.1989). A finding of an intent to deceive may follow from an assessment of materiality, knowledge, and surrounding circumstances, including evidence of good faith. *Kingsdown Medical Consultants Ltd. v. Hollister Inc.*, 863 F.2d 867, 876, 9 USPQ2d 1384, 1392 (Fed.Cir.1988), cert. denied, 490 U.S. 1067, 109 S.Ct. 2068, 104 L.Ed.2d 633 (1989). The district court found no such intent, stating:

123 the record is devoid of any evidence that would establish deliberate knowing withholdings of any kind by Dr. Hewick or GI. Dr. Hewick was a credible witness who spoke carefully and candidly about his work ... There is no evidence that Dr. Hewick withheld any information he believed was material to the patent examiner.

124 Amgen, 13 USPQ2d at 1791. There is no clear error in this finding. Amgen raises no inequitable conduct issues that were not fully considered by the district court. We have reviewed the record and find no abuse of discretion on the part of the district court. This is also not an exceptional case.

III. OTHER ISSUES

125 In view of our conclusion that the district court erred as a matter of law in holding that claims 1 and 3 of the '195 patent are not invalid, we vacate the district court's holdings relating to infringement of those claims. We have considered the other arguments by counsel on both sides and find them to be without merit.

CONCLUSION

126 We conclude that the district court did not err in its findings that claims 2, 4, and 6 of

« up The '008 patent are valid and enforceable and have been infringed by GI, and that claims 1, 8, 23-27, and 29 of the '008 patent are invalid; we therefore affirm the judgment of the court regarding the '008 patent. Because we conclude that claims 1, 3, 4, and 6 of the '195 patent are invalid, we affirm the judgment concerning claims 4 and 6 and reverse the judgment concerning claims 1 and 3.

COSTS

127 Each party shall bear its own costs.

128 AFFIRMED-IN-PART, REVERSED-IN-PART, VACATED-IN-PART.

¹ The district court, in a detailed opinion, fully sets out the scientific and historical background relating to the patents at issue. See Amgen, 13 USPQ2d at 1741-58. Familiarity with that opinion is presumed

² Amgen subsequently filed a complaint with the United States International Trade Commission alleging that Chugai's importation of rEPO, manufactured in Japan using genetically engineered host cells, violated Section 337 of the Tariff Act of 1930 (19 U.S.C. Sec. 1337, 1337a). The Commission entered an order terminating the investigation for lack of subject matter jurisdiction. This court vacated and remanded, holding that the Commission should have treated the complaint on the merits and not on jurisdictional grounds, and that the claims of Amgen's patent did not cover a process for producing rEPO. Amgen, Inc. v. United States Int'l Trade Comm'n, 902 F.2d 1532, 14 USPQ2d 1734 (Fed.Cir.1990)

³ We note that both the district court and the parties have focused on the obviousness of a process for making the EPO gene, despite the fact that it is products (genes and host cells) that are claimed in the patent, not processes. We have directed our attention accordingly, and do not consider independently whether the products would have been obvious aside from the alleged obviousness of a method of making them

⁴ At this point, some explanation of the involved technology may be useful, consistent with that expressed in the district court opinion. DNA consists of two complementary strands of nucleotides, which include the four basic compounds adenine(A), guanine(G), cytosine(C), and thymine(T), oriented so that bases from one strand weakly bond to the bases of the opposite strand. A bonds with T, and G bonds with C to form complementary base pairs. This bonding process is called hybridization and results in the formation of a stable duplex molecule. The structure also includes 5-carbon sugar moieties with phosphate groups

The genetic code for a particular protein depends upon sequential groupings of three nucleotides, called codons. Each codon codes for a particular amino acid. Since there are four nucleotide bases and three bases per codon, there are 64 (4x4x4) possible codons. Because there are only 20 natural amino acids, most amino acids are specified by more than one codon. This is referred to as a "redundancy" or "degeneracy" in the genetic code, a fact that complicates and renders more difficult the techniques of recombinant DNA.

In order to prepare a protein using recombinant DNA technology, the gene for the protein must first be isolated from a cell's total DNA by screening a library of that cell's DNA. The DNA library is screened by use of a probe, a synthetic radiolabelled nucleic acid sequence which can be used to detect and isolate complementary base sequences by hybridization. To design a probe when the gene has not yet been isolated, a scientist must know the amino acid sequence, or a portion thereof, of the protein of interest. Because some amino acids have several possible codons and the researcher cannot know which of the possible codons will actually code for an amino acid, he or she may decide to design a set of probes that covers all possible codons for each amino acid comprising the protein, known as a "fully-degenerate" set of probes. A library to be screened can be a genomic library (gDNA), which contains a set of all the DNA sequences found in an organism's cells or a complementary DNA (cDNA) library, which is much smaller and less complex than a gDNA library, and is used frequently when the tissue source for a given gene is known.

« up ⁵ Defendants assert that all the claims should be invalid for failure to disclose the best mode. We perceive that the best mode issue only relates to the host cell claims, 4, 6, 23-27, and 29. Absent inequitable conduct, a best mode defense only affects those claims covering subject matter the practice of which has not been disclosed in compliance with the best mode requirement. See Northern Telecom, Inc. v. Datapoint Corp., 908 F.2d 931, 940, 15 USPQ2d 1321, 1328 (Fed.Cir.), cert. denied, --- U.S. ----, 111 S.Ct. 296, 112 L.Ed.2d 250 (1990)

⁶ In its opinion, the district court stated that "the best way to express EPO was from mammalian cells ... and that a cell line derived from 11 possible clones from the CHO B11 3,1 cell strain was to be used for Amgen's master working cell bank, which was expected to be started on November 26, 1984." 13 USPQ2d at 1772. At another point, the court stated that Amgen "did disclose the best mode in Example 10 of the invention, when it described the production rates of the 100 nanomolar-amplified cells (the B11 3,1 cell strain) and one micromolar-treated cells." Id

⁷ See also 53 Fed.Reg. 39420, 39425 (Oct. 6, 1989) (comment re "deposit [to] satisfy the best mode requirement"); 52 Fed.Reg. 34080, 34080 and 34084 (Sept. 8, 1987) (deposit may be required to satisfy enablement, best mode, or distinct claim requirements of Sec. 112)

⁸ Cf. Hormone Research Foundation, Inc. v. Genentech, Inc., 904 F.2d 1558, 15 USPQ2d 1039 (Fed.Cir.1990). In Hormone Research, this court, in a remand, directed the district court to consider the effect of United States Steel Corp. v. Phillips Petroleum Co., 865 F.2d 1247, 9 USPQ2d 1461 (Fed.Cir.1989) and In re Hogan, 559 F.2d 595, 194 USPQ 527 (CCPA 1977) on Fisher in its enablement analysis. The facts of our case are distinguishable from those in Hormone Research, United States Steel, and Hogan

⁹ The probes designated "EpV" were from EPO amino acid sequence region 46-52

¹⁰ The potency of EPO in the '195 patent is stated as its specific activity, expressed as a ratio of International Units (which measures the ability of EPO to cause formation of red blood cells) per absorbance unit (the amount of light absorbed by a sample of EPO measured by a spectrophotometer at a given wavelength, 280 nanometers), i.e., IU/AU

¹¹ Defendants provided no evidence that faulty purification procedures or other missteps caused its failure to obtain 160,000 IU/AU in vivo material as claimed in the '195 patent

¹² The court quoted Chugai to the effect that the in vivo activity of uEPO is 65 percent that of rEPO

¹³ Chugai's sample reported to the Food and Drug Administration was not purified by the disclosed process



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